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Canine creatinine plasma concentration control, renal clearance and compartmental analysis

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CANINE CREATININE PLASMA CONCENTRATION CONTROL, RENAL CLEARANCE AND COMPARTMENTAL ANALYSIS

by

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PREFACE

This thesis is submitted to the Department of Physiology of the Yale Medical School as partial fulfillment of the requirements of the Doctor of Medicine Degree. It describes experiments performed at the University of Minnesota in the summer of 1964. The computer data analysis and simulation were carried out at the Mayo Clinic in Rochester, Minnesota during the summers of 1964 and 1965.

It is my pleasure to acknowledge the assistance and guidance of Rodney B. Harvey, M.D., Ph.D., and associate professor of physiology at the University of Minnesota. The animal experiments were performed in his laboratory and the control system was basically of his design.

James B. Bassingthwaighte, M.D., Ph.D. and member of the staff of the department of physiology at the Mayo Clinic also deserves my special thanks. It was at his request that this project was initially undertaken and his assistance, particularly with the computer analysis and simulation, has been invaluable.

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Finally, I thank the numerous helpful technicians at the Mayo Clinic and University of Minnesota. They not only helped to perform the experiments, but also assisted in the analysis of the data and the preparation of the figures in the text.

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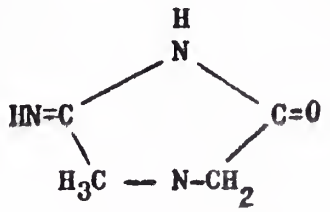
INTRODUCTION

The project dexcribed in this thesis addressed itself to the solution of three separate, but related, major problems concerned with the physiology of creatinine in the dog. Creatinine is the anhydride of creatine and has the following structure:



It is formed by the non-enzymatic, irreversible dehydration of creatine phosphate in muscular tissue (73). The liver is the major source of creatine (26,111). Creatinine is a nontoxic, diffusible compound that is removed almost exclusively by the kidney. A primary objective of this study was to measure the dog's glomerular flitration rate by a new technique that eliminated the errors introduced by collection of urine samples. A second goal in this research was to perfect a servo-controlled, automated system that was capable of both monitoring and maintaining the plasma creatinine concentration at a pre-selected level. The third major objective in the research presented here was to formulate a theoretical model, based on compartmental theory, which was capable of describing the dynamics of the distribution of creatinine in the dog's body. The following chapters are devoted to a discussion of these three problems and how they relate to a better understanding of the various fields of renal function, physiological control, compartmental analysis, and computer applications to physiology.

The fundamental relationship which forms the basis for much of this work is the assumption that the rate of infusion of a non-metabolized substance such as creatinine must be equal to the sum of the rate of excretion and the rate of accumulation of the substance in the body:

$$\frac{d(Q_{infused})}{dt} = \frac{d(Q_{excreted})}{dt} + \frac{d(Q_{tissues})}{dt} \qquad (1)$$

This relationship is merely a restatement of the law of conservation of matter. A similar relationship has been used by various investigators (12,41,60,65,84,89,96,140) who have assumed that when an equilibrium has been reached (i.e. $\frac{dQ_{tissues}}{dt} = 0$) the rate of infusion of a non-metabolized substance is equal to its rate of removal by the kidney.

For the purposes of the present study, creatinine may be considered a non-metabolized compound since the quantities involved in these experiments were far in excess of the endogenous creatinine production and extra-renal creatinine removal. The rate of endogenous creatinine production has been found, in normal dogs, to be 0.35mg./min.(65). In nephrectomized dogs, the rise in creatinine plasma concentration due to endogenous creatinine production has been found to be 0.14mg.%/hour(139) and 0.11mg.%/hour to 0.27mg.%/hour (171). Edwards (44) demonstrated that creatinine concentration increased only 1mg./liter/hour in the plasma of anuric rabbits. Lodell (91) found that negligible amounts of creatinine are removed in the sweat and saliva. Maw (108) found that 85% of ingested creatinine appeared in the urine and 15% was found in the feces. By isotopic techniques it has been shown (79) that the rate of turnover of the total body creatinine and creatine is about 1.6% per day. From the above data it seems justified to neglect the amount of creatinine produced by the animal and the amount removed by extra-renal

routes during the experiment. The endogenous creatinine that is present in the dog at the beginning of the experiments will be considered in the analysis of the experimental results.

The fundamental Equation (1) was applied to experiments performed in nine mongrel dogs. The utility of this relationship in the solution of the three major problems mentioned above is the topic of the three following chapters. A closed-loop control system has been developed which allows the investigator to maintain the plasma creatinine concentration, $[Cr]_p$, at an elevated and nearly constant level. To achieve this control an automated chemical method was perfected for constantly measuring the dog's $[Cr]_p$. This measured concentration is used by a newly developed electrical servo-mechanism to produce an error signal proportional to the difference between the desired and the measured $[Cr]_p$. A variable speed infusion pump, which discharges a concentrated creatinine solution directly into the dog, is driven at a rate proportional to the error signal generated by the servo control mechanism. As demonstrated in Chapter I, the plasma creatinine concentration was controlled by this feedback system. In some experiments inulin was also added to the infusion solution and the plasma concentration of this substance was indirectly controlled by this system.

The volume of the solution pumped into the dog was recorded continuously by a newly developed volume meter. Thus, the first term in Equation (1) could be accurately determined. A comparison of this measured rate of infusion to simultaneously measured rates of creatinine excretion in the urine established that under certain circumstances, one may measure the rate of urinary excretion without collecting urine. Chapter II is devoted to the presentation of data pertaining to this

indirect measure of kidney function.

Finally, Chapter III presents a computer simulation of the animal experiments described in Chapter I. This simulation incorporates mathematical models of both the chemical creatinine sampling system and the experimental animal. The accuracy of the sampling system simulation model was verified by its ability to reproduce faithfully the time lag and distortion of the input signal that was actually observed in the experimental chemical sampling system. For the purposes of this simulation, the experimental animal was considered to consist of a series of separate and well-mixed compartments into which the creatinine is distributed. The differential equations that describe the dynamics of the creatinine distribution were formulated according to compartmental analysis theory (16,92,131,149,152,156). These simultaneous differential equations were solved by means of high speed digital and analog computers. A voltage representing the observed rate of creatinine infusion was used as the driving function for this simulation. The accuracy of the compartmental representation was tested by its ability to predict the plasma creatinine concentration that was actually observed in the experimental animal. The physiological significance of this kinetic analysis and of the various compartments in the mathematical model of the dog are discussed in Chapter III.

CHAPTER I

REGULATION OF THE PLASMA CREATININE CONCENTRATION IN THE DOG USING A SERVO CONTROL SYSTEM

A. Purposes, Review of Literature and General Description.

The measurement and control of biological phenomena are among the primary objectives of the physiologist. The classical method of attaining these ends is to (a) compensate for changes in all variables in the system, (b) allow the parameters of interest to vary in an orderly manner and (c) measure the effects of this variation in the system. In the present study, a technique is presented which changes the emphasis, but not the the basic principles, of this classical research method. Here the emphasis is placed upon control. The adjustments that are made by the controlling system are measured. These adjustments are, in turn, analyzed in order to elucidate the physiological mechanisms of the process under study.

A system has been developed which is capable of maintaining the plasma creatinine concentration at an elevated, nearly constant level. This system was primarily designed in order to increase the accuracy of renal clearance measurements. It is to be recalled that in calculating the clearance of a compound from the body, it is necessary to know the plasma concentration of that substance. In most urinary clearance demonstrations, the material under study is injected into the animal and urine is collected at regular intervals. Blood samples are also drawn at the mid-point of each of the urine collection periods. The plasma concentration of the material under study at the mid point of the urine collection period is used to calculate the urinary clearance during that interval (39,89, for example). It is obvious, though, that while the injected compound is being removed from the body, the plasma con-

centration is constantly changing, so that a single plasma level determined at the mid point of the interval is not always an accurate reflection of the concentration during the entire urine collection period. This introduces an error into the calculated clearance. It is apparent that a continuous measurement of the plasma level of the compound under study would at least allow one to determine the average plasma concentration during the urine collection period and therefore yield more accurate clearance calculations. The accuracy of using this average plasma concentration in calculating the urinary clearance is limited, however, by the imprecision of matching the urinary collection interval with the corresponding interval during which the blood concentration is to be averaged. This imprecision is introduced by the various time delays involved in the flow of urine through the collecting system of the urinary tract. If the plasma concentration of the substance under study were maintained constant during the entire urinary collection interval, it seems that a more accurate calculation of the urinary clearance would be possible. This would eliminate the error involved in using mid point and average plasma concentrations and also allow the investigator to determine accurately the relationship between the plasma concentration and the urinary clearance of a particular substance. This is particularly important when one is studying the clearance of substances that are either secreted or reabsorbed by the kidney because in these situations, the urinary clearance changes as a function of the plasma concentration. Teschen (162) has discussed the errors involved in clearance calculations in subjects with changing blood concentrations and emphasizes that some of the basic assumptions of the clearance calculation come under suspicion in these circum-

stances. Therefore, a system to maintain a constant plasma creatinine concentration was constructed to facilitate accurate renal clearance measurements.

A second motive for designing this control system was to ascertain the capabilities, operating characteristics and limitations of this type of regulatory mechanism. As mentioned before, physiologists have traditionally been interested in both naturally occurring control mechanisms as well as artificial means of regulating physiological parameters. Virtually every field of physiology, particularly respiration (34), temperature regulation (72), and circulation (167) and neurophysiology (10) has stimulated research into the complex natural mechanisms that maintain homeostasis. This study has been greatly facilitated by the application of electronic apparatus simulating postulated natural regulatory mechanisms (10). Aside from applications to research devoted to the understanding of natural homeostatic mechanisms, control apparatus of the type described here could have clinical application in situations where the normal regulatory mechanisms are altered by disease. Mechanical and electrical control apparatus has already been applied to regulate the heart rate by artificial pacemakers, and to control the body temperature. One can also postulate that in addition to growing research and clinical applications, electronic control of physiological variables will become more important as man invades the alien environments of the deep sea and outer space. The present study, then, was undertaken to investigate the properties of a new control apparatus that might serve as a prototype for equipment that could be applied to other research studies, clinical situations, and technological problems.

Other investigators have been interested in artificially controlling physiological variables. Kadish (87) has regulated the blood sugar concentration in two patients using a closed-loop feedback system that incorporated the continuous measurement of the blood sugar level and the infusion of either insulin or a concentrated glucose solution. This control was not precise, due mainly to the seven minute time delay introduced into the controlling circuit by the chemical analysis of the blood sugar. There were wide oscillations in the blood sugar concentration. Previous attempts (65,84,89) to regulate the plasma creatinine concentration have involved the continuous infusion of a concentrated creatinine solution. This maintains the creatinine concentration at a constant level determined by the rate of infusion and rate of renal excretion. This particular method was first applied by Earle and Berliner (41) to the study of inulin excretion and has subsequently been applied to maintain a constant plasma concentration of mannitol (96,140) para-amino hippuric acid (12,41,65) and inulin (12,60,84,89). This general method of constant infusion has the disadvantage that the steady state plasma concentration is partially determined by the rate of renal excretion. Small changes in renal function perturb the constant plasma concentration. The system described below offers the advantage that the investigator can pre-select the desired plasma concentration and this level can be maintained almost indefinitely and virtually independently of the renal status of the experimental subject.

This controller is basically a closed-loop, direct feedback, continuous servo control system. Bayliss has said (10), "It is the presence of the feedback element, the introduction of a 'closed-loop' and the activation of the motor by the misalignment between input and output that are characteristic of a 'servo system'." Reference to

Figure 1 will demonstrate that the closed loop is composed of the experimental animal, a continuous chemical plasma creatinine detector, an electronic control mechanism and a variable speed infusion pump. The investigator sets the electronic controller to maintain the desired plasma creatinine concentration. This controller generates a continuous electric signal which is proportional to the difference between the desired $[Cr]_p$ and the creatinine concentration actually observed in the experimental subject at that instant. This signal determines the armature current for a variable speed motor driving a pump which infuses a concentrated creatinine solution into the experimental animal. The continuous chemical analysis by means of the Technicon AutoAnalyzer* system forms the feedback element in the closed loop. Thus, the rate of infusion is altered as the $[Cr]_p$ changes and control is thereby achieved. It is emphasized that the two factors which tend to lower the $[Cr]_p$, namely diffusion into the animal's tissues and excretion in the urine, are integral parts of the control system.

Due to the fact that there are unavoidable delays involved in this feedback element, oscillations in the regulated $[Cr]_p$ are present. These delays arise mainly in the time involved for creatinine to circulate from the animal's femoral vein to femoral artery in addition to the time necessary for the completion of the chemical analysis. The modifications that were incorporated into this system in order to minimize these delays will be described in the following sections, which present in more detail the characteristics of the control system components.

*Registered trade name

B. Continuous Measurement of Plasma Creatinine Concentration

An essential aspect of the control system was the continuous measurement of the plasma creatinine concentration. In the literature there is a wide variety of methods for measuring creatinine and it is valuable to discuss these techniques briefly, in order both to justify the choice of measuring creatinine that was used in these experiments and also to establish that many of the previous methods for this measurement have subsequently been shown to be invalid. Most of these techniques are based on the colorimetric estimation of the red-colored end product of the reaction between creatinine and alkaline picrate. This method was first described by Jaffé (83) in 1886 and was popularized by Folin (52,53) and used by Bauman and Hines (9) to measure the creatinine in tissues. It soon became evident that this technique gave high false values when used to measure creatinine (46,55). There are other chromagens, particularly protiens, urea, formaldehyde and guanidine compounds that also react with the picrate and give a red color (3, 27,38,94,100). To improve the Jaffé method, a spate of procedures has been proposed which purport to measure the "true creatinine" by separating the creatinine from the cross-reacting chromagens before reacting it with the alkaline picrate. The Folin-Wu method (55), which involves adsorption of creatinine in Lloyd's reagent, has become popular in many clinical laboratories (7,163) in spite of serious doubts of the accuracy of this method (46). Protiens have been removed by precipitation with trichloroacetic acid (89,104), by tungstate (39,55,112,133), by zinc oxide (3) and by dialysis (27) before applying the Jaffé reaction. The smaller cross-reacting chromagens have been removed by

evaporation (40), extraction (110), reduction (163), resin exchange (127) and papee chromatography (107). It is apparent that the Jaffé reaction suffers from lack of specificity and there is no modification of this method that clearly surmounts this shortcoming.

Perhaps the most specific method for measuring the creatinine in biological fluids is an enzymatic technique described by Miller et al. (112,113). From an organism selected to grow on creatinine as its only source of carbon and nitrogen they isolated specific enzymes that metabolized creatinine. The "true creatinine" is measured as the difference in the alkaline picrate reactive substance determined before and after treatment with these enzymes. Creatinine has also been measured using N^{15} labelled creatinine and measuring the radio-activity (79). A nephelometric method using modified Nessler's solution and measuring the light scattered from a creatinine suspension has been claimed (3) to be quite specific, but due to the difficulty of the procedure this technique has not become popular.

In the present study, the creatinine concentration was measured continuously by the Technicon AutoAnalyzer. The chemical method used for this purpose was suggested almost simultaneously by Benedict and Behre (11), Langley and Evans (94), and Bolliger (20). In this analytic technique, the color developed by the reaction of creatinine with alkaline 3, 5 dinitrobenzoic acid is measured photometrically. This chemical method was selected for the present study because it is more specific for creatinine than the Jaffé reaction (11,94,134). It is believed (3,112,113) that even with the complex precipitation and extraction techniques, there is chromagenic material remaining in the biologic fluid so that the alkaline picrate reaction gives falsely high

estimates of creatinine. This is not the case with the 3,5 dinitrobenzoic acid method and therefore the estimates of creatinine made by this method are closer to the "true creatinine". The benzoate method is, however, less sensitive than the picrate method (3,94) and is not accurate below 0.3mg.%. This limitation is of little importance in the present study because the creatinine concentrations were usually in the range of 10 to 40mg.%. The major source of error in the 3,5 dinitrobenzoate method is that the color tends to be unstable (104). This source of error was virtually eliminated, however, in the adaptation of this method to the AutoAnalyzer because in this machine the color is always read at precisely the same time.

Previous attempts to measure creatinine with the AutoAnalyzer have all used the Jaffé reaction. Unfortunately, three of these methods (19,125,173) did not incorporate steps to remove the cross-reacting chromagen from the samples and therefore suffer from the lack of specificity discussed above. Polar and Metcoff (127) measured the creatinine in plasma samples after the specimens had been manually passed over an ion exchange resin. Chasson et al. (27) describe an automatic creatinine analysis which applies the Jaffé reaction to a dialysate produced by the AutoAnalyzer. Blood sugar and PAH are the only compounds which heretofore have been measured continuously in experimental subjects (50,70,87,170).

The AutoAnalyzer was arranged as presented in Figure 2. The color reagent was prepared by adding 132 grams of 3,5 dinitrobenzoic acid (Fischer #A-114) to an aqueous solution of 54 grams of Na_2CO_3 and two ml. of Brij-35 (Technicon Chemical Corporation). The volume was adjusted to 4 liters with distilled water and the pH was reduced to

7.0 ± 0.05 using concentrated HCl. It was necessary to warm and stir vigorously this solution to eliminate the excess CO_2 during the pH adjustment.

Aqueous creatinine standards in the range of 0-40mg.% were useful in determining the relative sensitivity, baseline stability and drift of the AutoAnalyzer system. Consecutive samples of the same 20mg.% creatinine solution resulted in deflections on the AutoAnalyzer chart which differed by less than one percent (i.e. 0.2mg.%).

In order to determine the creatinine concentration in the plasma of whole blood samples, the system was calibrated using blood in which the plasma concentration had been measured. The whole blood samples were centrifuged and the plasma creatinine concentrations were measured by an AutoAnalyzer which had been calibrated with aqueous creatinine solutions. Direct calibration of the whole blood samples using standard aqueous creatinine solutions gave low, incorrect values for the plasma creatinine concentration. This is due to the fact that the creatinine moves in and out of the cells very slowly. The half-time for creatinine to move from plasma into the erythrocyte has been found by Van Slyke (165) to be on the order of one hour. At a constant sampling rate, therefore, whole blood presents less dialyzable material to the dialysis membrane than does a pure aqueous solution. This results in less creatinine crossing the membrane from whole blood of a given plasma concentration than from an aqueous solution of identical concentration. Using plasma to calibrate the whole blood creatinine determination, the reading on the linear AutoAnalyzer recorder was observed to be directly proportional to the plasma creatinine concentration of the whole blood sample. In practice, the variation of the hematocrit that occurred

during the experiments did not significantly change the calibration curve obtained as described above. Figure 3 presents a calibration curve obtained for one experiment.

Adequate control of the $[Cr]_p$ required that the time delay involved in the measurement of the $[Cr]_p$ be minimized. Several features were incorporated in order to reduce this delay. First, sampling from an artificial arterio-venous fistula eliminated the delay introduced by the transfer of the arterial sample from the animal to the dialyzer. Second, passing the blood through the dialyzer before the pump eliminated the time delay in the passage of the arterial sample through the pump. Third, the high flow rate of the recipient stream in the dialyzer reduced the time involved in moving the sample to the colorimeter. Fourth, heating the coil in which the color developed increased the rate of the color reaction. It has been previously demonstrated that heating creatine in the presence of 3,5 dinitrobenzoic acid does not convert it to creatinine (94). Fifth, all tubing involved in the transmission of the sample was cut as short as possible. As shown in Figure 4, the AutoAnalyzer recorder pen was observed to begin to respond 1.1 minutes after the sample was introduced at the sampling site. The mean transit time for a 20mg.% sample was 1.9 minutes. The shape of this curve was the same in response to step changes in the creatinine concentration regardless of the magnitude.

The pen recorder connected to the AutoAnalyzer was modified in two respects. First, the recorder was modified so that the pen deflection was linearly rather than logarithmically related to the color of the solution in the colorimeter cell. This modification was introduced in order to make the control system response more directly proportionally

related to the $[Cr]_p$. Second, a retransmitting 5000 ohm potentiometer was mechanically linked to the recorder slide wire. This potentiometer provided the input signal to the electrical servo control mechanism.

C. Servo Controller and Infusion System

Reference to Figure 5 reveals that the servo controller used in the present study had three sources of input information. The first was the position of the pen on the AutoAnalyzer recorder that was transmitted by the 5000 ohm potentiometer attached to the recorder slide wire. The second means of input was the Span Switch #1 which determined the range of pen position around the desired position that the mechanism would act as a proportional controller. If the pen were above the range of the controller, the infusion pump is turned off; if the pen were below the range determined by the Span Switch, the pump is driven at the maximum rate. When the pen is within the range of the servo controller, the pump is driven at a rate approximately proportional to the position of the pen on the AutoAnalyzer chart. Switch #1, then, is used to control the throttling range of the proportional controller and if the pen is outside of the range of the servo controller, the system responds like an on - off regulator. At one extreme setting of the Span Switch the servo mechanism acted as a proportional controller over much of the Auto Analyzer chart. In this extreme position, the controller tended to respond slowly to variations in the pen position and the entire system was therefore over-damped. At the other extreme setting of the Span Switch, the controller responded in a proportional manner over only a small segment of the AutoAnalyzer chart and the system

was then essentially an on - off controller. The setting of the Span Switch was changed occaisionally during the experiments in order to obtain the control characteristics desired. The third route of input information to the controller is the Potentiometer #2 represented on Figure 5. This control determines the set point, or pen position on the AutoAnalyzer recorder at which the plasma creatinine concentration will be maintained. This set point was continuously variable over the entire range of the recorder. Thus, both the desired $[Cr]_p$ and the type of regulation could be adjusted using control knobs #2 and #1 respectively.

The servo controller determined the amount of current supplied to the armature of the pump used to deliver the concentrated creatinine infusion solution into the experimental animal (Figure 5). The pump was powered by an 80-100 volt DC motor. It was equipped with 12 rollers which compressed a flexible tube containing the infusion solution. When a tube of 0.081 inches inside diameter was used in the pump, the maximum rate of infusion was 30 ml./min.. Additional change in the infusion rate was achieved by changing the gear ratio between the pump motor and the rollers. For this purpose, variable gears were placed between the pump and the motor which allowed the gear ratio to be adjusted by a factor of 8 in 4 steps. An initial rapid infusion rate was desired in order to achieve a step change in the plasma creatinine concentration and this was accomplished using a large motor:roller pump gear ratio. Later in the experiment when the creatinine was to be infused more slowly, the gear ratio was reduced to minimize the oscillations in the $[Cr]_p$.

The infusion solution was prepared by dissolving 10 or 15 grams of reagent grade creatinine and 234 grams of mannitol in distilled water and adjusting the total volume to two liters. The creatinine concen-

tration of each infusion solution was determined colorimetrically. The mannitol was added to the infusion solution in order to encourage an osmotic diuresis so that the urine volume would be adequate for the accurate determination of the urinary clearances.

The volume of fluid infused into the animal was measured by a volume meter consisting of a vertical three feet section of plastic pipe (0.6 inch inner diameter) with a Statham pressure transducer connected at the bottom. The fluid infused into the experimental animal is pumped from this reservoir. The output voltage of the transducer is directly proportional to the height of the fluid in the column, and is therefore proportional to the volume of the fluid in the column. This voltage was amplified by a Carrier amplifier and photographically recorded on a Hieland oscillograph. This volume meter was calibrated by adding successive 20.0 ml. aliquotes of the infusion solution to the column. This volume meter was found to be remarkably stable and reproducible and could be read with accuracy to 0.5 ml. in 150 ml. The rate of infusion was also read from the volume meter record by measuring the slope of the graph of the volume recorded as a function of time.

D. Indirect Control of the Plasma Inulin Concentration

It was possible to control indirectly the plasma inulin concentration using the system described above. In order to achieve this, a fixed relationship must exist between the excretion of the substance controlled directly by the servo system and the compound indirectly regulated. It has been amply demonstrated that the clearances of crea-

tinine and inulin are essentially identical (46,68,69,84,89,120,142,145). Inulin is a polysaccharide that is neither created nor destroyed in the body. $\frac{dQ_{excreted}}{dt}$ from Equation 1 is therefore equal for both creatinine and inulin. Thus, when inulin is infused at the same rate as creatinine, the plasma concentration of inulin will be maintained at as constant a level as the creatinine plasma concentration after the tissue stores of creatinine and inulin are saturated i.e. $\frac{dQ_{tissues}}{dt} = 0$. The exact level of the inulin plasma concentration will be determined by both the rate of urinary inulin excretion and the concentration of inulin in the infusion solution. In four experiments, tritiated inulin (New England Nuclear Co.) was added to the infusion solution. Inulin concentrations were measured on urinary and plasma samples using liquid scintillation counting techniques.

E. Preparation of Dogs and Experimental Protocol

The servo system described above has been used to control the plasma creatinine concentration in nine mongrel dogs. In four dogs, the plasma inulin concentration was simultaneously controlled by the indirect manner previously described. The protocol for these experiments was similar in all instances. The dogs were weighed and anesthetized with 4% sodium pentobarbital (Nembutal). The right femoral vein was cannulated for the infusion of the creatinine solution. The right femoral artery was also cannulated for monitering the blood pressure and withdrawing arterial blood samples. The volume meter was calibrated while the reservoir was filled with 20 ml. aliquotes of the infusion solution. The bladder was catheterized and emptied. The left

femoral vein was cannulated with polysthylene tubing (0.11 inch inner diameter). These two cannulae were joined with a glass "h" connector forming an arterio-venous fistula. The animal was heparinized and the femoral artery-femoral vein fistula was connected to the AutoAnalyzer dialyzer for continuous whole blood sampling. The set point and span controls were set to the desired positions and this initiated the creatinine infusion. When the infusion pump began, a timer was started. Five minutes after the creatinine infusion began, 4 ml. samples were drawn at ten minute intervals from the right femoral artery. These blood samples were centrifuged and the plasma used for calibrating the AutoAnalyzer as described above. Every ten minutes the urine was removed from the bladder and in some animals the bladder was washed with 30 ml. of 0.9% saline. The wash solution and the urine samples were mixed for the purpose of the analysis. Creatinine in the urine was measured by an AutoAnalyzer technique similar to that used for measuring $\left[Cr\right]_p$, except that the method was calibrated using standard aqueous creatinine solutions. During the course of the experiment, it was necessary to refill the volume reservoir with the creatinine infusion fluid. The gear ratio on the infusion pump was reduced as the experiment progressed in order to minimize the oscillation in the $\left[Cr\right]_p$. The experiments were continued from 160 to 200 minutes.

F. Technique of Data Analysis

The data from the experiments on the dogs were analyzed on an IBM 1620 computer. A calibration program was written to convert the AutoAnalyzer and Hieland records to concentration and volume units,

respectively. The pen deflection was read visually at twenty second intervals from the AutoAnalyzer chart. The calibration curve for the AutoAnalyzer was constructed by pairing the plasma creatinine concentration from each of the 4 ml. arterial blood samples with the AutoAnalyzer chart reading occurring two minutes after the blood sample was collected (to account for the delay time of the AutoAnalyzer system and the dog). The best calibration curve was computed on the IBM 1620 by the method of least squares. Correlation coefficients of 0.98 were routinely obtained for this calibration. From this calibration line, the 1620 computed the plasma creatinine concentration at each one-third minute during the experiment.

Similarly, the deflection on the Hieland record was measured in centimeters for each 20 ml. aliquote of infusion solution added to the volume meter column at the beginning of the experiment. By matching the volume of fluid in the column with the measured deflection on the Hieland record, a calibration curve was constructed. The best calibration line was computed by the method of least squares. Correlation coefficients of 0.99 were usually computed for the volume meter calibration. The Hieland record made during the experiment was also measured at twenty second intervals and the volume of infusion solution in the column was then computed using the volume meter calibration line. In this program, provision was also made for computing by interpolation the volume infused during the time in the experiment when the infusion column was being refilled. Finally, the calibration graphs as well as creatinine concentration and total volume of creatinine concentration and total volume of creatinine infused as a function of time were plotted using a Calcomp on-line plotter (California Computer

Products, Anaheim, California). The calibration program also entered the computed concentrations and volumes into punched cards. These cards were used as input data for further computer analysis as described in succeeding chapters.

G. Results and Discussion

In the nine dogs used in these experiemtns, the servo control both attained and maintained the desired plasma creatinine concentration. When the controller is turned on, the system attempts to regulate plasma creatinine concentration at a new level, as is shown in Figure 6. Following an initial "overshoot", the plasma creatinine concentration oscillates about the desired level. These oscillations tended to be damped. In spite of the continued fluctuation, the average concentration over successive ten minute intervals remained nearly constant.

The oscillations in the plasma creatinine concentration are the result of the delays present in the system. The times involved in the chemical analysis and in the circulation from the dog's femoral vein to the femoral artery are the major sources of this delay. The small delays, due to the recorder pen response and to the recorder-servo control mechanical linkage and to the response of the infusion pump, are negligible. The period of these oscillations is about five minutes, which is approximately twice the mean transit time of the entire systems. The oscillations can be expected to be increased as the system delay is prolonged or as the infusion fluid creatinine concentration is elevated. It is apparent, however, that as long as there are delays in this system, oscillations, however slight, will be present, unless the system is severely overdamped. At any instant,

the servo controller is responding to the $[Cr]_p$ that existed in the animal at an earlier time, which is equal in magnitude to the sum of the delays in the system. Thus, when the $[Cr]_p$ is rising, a delay in the servo controller's recognition of this increase results in an overly rapid infusion of creatinine into the dog. This leads to an "overshoot" in the $[Cr]_p$. Similarly, when the pump is turned off, as a result of this "overshoot", the plasma creatinine concentration begins to fall. But, since this is belatedly recognized by the servo controller, the $[Cr]_p$ falls below the desired level, resulting in an "undershoot" in the plasma creatinine concentration.

In the present study, the amplitude of the oscillations was reduced by adjusting the motor:pump transmission gear ratio. In the initial phase of the experiment, a rapid step-like change in the plasma creatinine concentration was desired. This required a high motor:pump gear ratio. Later, when the minimization of the oscillation in the $[Cr]_p$ was a major consideration, this gear ratio was reduced to prevent the infusion of excessive creatinine during the delay period. Figure 7 presents the relationship between the plasma concentration and infusion rate computed at twenty second intervals for various gear ratios during one experiment. This represents the transfer function of the servo control infusion system. It is apparent that at lower gear ratios, the maximum rate of infusion is less than that for higher gear ratios. A practical reason for decreasing the gear ratios was that the infusion rate required late in the experiment (when it is compensating primarily for the renal excretion rate) was only 1 to 2% of that required initially (when the infused creatinine was moving rapidly into the tissues).

The behavior of this control system may be better understood by referring to Figure 8. The heavy line, ABCDEF, represents the relationship between the infusion rate and plasma creatinine concentration. This is a fixed relationship for a given experimental arrangement, provided there is no drift in any of the control system components. The straight line labelled "Renal Clearance = 100 mL/min." is one of a family of lines that represents the rate of creatinine removal, by way of the kidney for any given concentration. For substances cleared by glomerular flitration, this line will be straight. When the servo control system is operative, there will eventually be an equilibrium attained between the blood and tissue concentrations, and a steady state relationship will exist between the pump infusion rate and renal clearance. This situation is represented by point E in Figure 8.

At the beginning of the experiment, the plasma creatinine concentration is low and therefore, the infusion rate will be maximal, near point A. If there were no delays in the AutoAnalyzer system or in the dog, then the plasma creatinine concentration would increase and the infusion rate would decrease along the line ABCDEF. In other words, the line labelled infusion clearance would rotate clockwise until point E was reached. Point C, which represents the rate of creatinine infusion and the rate of creatinine removal from (and to some extent, dilution in) the blood, moves gradually along the line ABCDEF, until diffusion into the tissues is negligible. Points C and E then coincide and the infusion rate is balanced only by the renal excretion rate. When delays are present within the system, however, oscillations are introduced as described above. Then point C moves past point E to D or F and then returns along the same path.

An appreciation of the degree to which the plasma inulin concentration was indirectly controlled can be obtained by referring to Figure 9, The plasma inulin level was measured approximately every ten minutes and is relatively constant, especially during the latter part of the experiment. The slight deviations from an absolutely constant plasma concentration are merely reflections of the oscillations in the plasma creatinine concentration.

In review, the exact type of control attained by the system used in this study is dependent on many factors. The set point controls the level at which the creatinine will be regulated. The Span control determines the range in which the controller response is proportional to the plasma creatinine concentration. The sensitivity of the AutoAnalyzer determines the plasma concentration that corresponds to a given deflection of the recorder chart. The delays involved in the system response contribute to the oscillations in the controlled plasma concentration. The concentration of the infusion solution also contributes to these fluctuations. The oscillations can be partially eliminated by adjusting the motor:roller pump gear ratio. The system described here was capable of maintaining a relatively constant $[Cr]_p$ in the animals in spite of oscillations. The plasma inulin concentration was also controlled indirectly. In order to maintain this type of indirect control, it is necessary that the inulin and creatinine be removed from the plasma in approximately the same manner. It may be concluded from this study that within the practical limitations discussed previously it is indeed possible to control the plasma concentration of a diffusible indicator, such as creatinine, by the servo system described here. The exact type of control obtained is subject to a wide number of variables (i.e. delays

in the system, excretion rates, analytical sensitivities) which must be considered in each instance when applying this type of control system to other indicators.

CHAPTER II

MEASUREMENT OF RENAL CLEARANCES WITHOUT COLLECTION OF URINE

A. Purposes, Theory and Equations

The servo system described in the previous chapter was used to measure the rate of renal creatinine excretion without collecting urine samples. The rate of urinary creatinine excretion is of considerable research and clinical interest, because it is a fairly good approximation of the glomerular filtration rate. The accepted measure of the glomerular filtration rate is the inulin clearance (60,126). It has been previously shown that in the dog (120,142,145), rabbit (84) and female rat (69), the urinary creatinine clearance is very nearly equal to the urinary inulin clearance. In man and guinea pigs, however, creatinine is secreted by the renal tubules and therefore the rate of indogenous creatinine clearance is not an accurate measure of the glomerular filtration rate (14,39,121). However, if exogenous creatinine is given to elevate the plasma creatinine concentration, the clearance of creatinine is a useful clinical approximation to the glomerular filtration rate (37,126). In view of the fact that the glomerular filtration rate reflects the clinical renal status and is also used to elucidate basic renal physiology, it is important to have a method which will accurately and easily measure this useful parameter.

The creatinine urinary clearance for a specific interval of time is calculated from the formula:

$$C_{cr}\ \frac{ml}{min} = \frac{\left[Cr\right]_u\ \frac{mg}{ml}}{\left[Cr\right]_p\ \frac{mg}{ml}} \cdot \dot{V}\ \frac{ml}{min} \qquad (2)$$

Here, $[Cr]_u$ is the urinary creatinine concentration, V is the ml of urine collected per minute during the interval. C_{cr} is the clearance of creatinine, which is the volume of blood "cleared" per minute of creatinine. The calculation of the clearance involves an accurate evaluation of each of the three factors in Equation 2. However, these measurements are subject to certain imperfections, as discussed below.

One of the drawbacks of using Equation 2 to calculate renal clearance is that the measurement of $[Cr]_u$ is impossible in anuric subjects and therefore the urinary clearance method is not applicable in certain circumstances. Also, the measurement of $[Cr]_u$ and $[Cr]_p$ often requires two separate techniques or standardizations, because, as compared to plasma, urine contains less cross-reacting chromagenic substance. The need to use two separate techniques can introduce serious systematic errors into the clearance calculations.

The previous chapter has presented a discussion of the errors involved in the accurate evaluation of the $[Cr]_p$ during clearance measurements. These are especially important when performed under conditions of changing $[Cr]_p$.

The evaluation of V also limits the accuracy of the classical creatinine clearance calculation. After the urine is formed by the tubules, it must pass through a collecting system before it can be sampled at the bladder. In dogs this dead space has a volume of about 6 ml. (60). A definite amount of time, $(t)_D$, is required for the formed urine to pass through this dead space. The exact magnitude of $(t)_D$ is dependent on the urine flow rate. However, when the clearance of a substance is measured over an interval $(t)_A$ to $(t)_B$, due to the dead space error, the urine collected in the bladder during this interval

was actually formed by the kidney during the period $(t)_A - (t)_D$ to $(t)_B - (t)_D$. Under conditions of low urine flow rates and varying plasma concentrations, the error introduced into the classical clearance calculation is considerable (60). The magnitude of this error can be reduced, but not eliminated, by a surgical cannulation of the renal pelvis. The correction for this dead time is especially important when one is studying the immediate effect of measures which rapidly change the filtration rate.

Another error inherent in the evaluation of the renal clearance by the urinary collection method is that all of the urine formed during a given interval must be quantitatively collected. This is difficult because there is usually some residual urine left in the bladder after voiding. In order to circumvent this source of error, it is necessary to catheterize the experimental subject and wash out the bladder with saline after each urine collection.

The present study was undertaken, therefore, to devise a method of measuring the creatinine clearance without introducing these errors inherent in the classical clearance technique. The study was undertaken also to measure the canine inulin clearance by a method that did not require the collection of urine. This clearance was to be compared with the creatinine clearance simultaneously determined.

The indirect method of measuring the creatinine clearance used here was based on the fundamental Equation 1. Under conditions in which the rate of creatinine accumulation in the tissues is zero, the rate of infusion by the servo controller is equal to the rate of renal excretion. The clearance can be easily calculated by dividing the rate of infusion by the plasma concentration. The clearance calculated in

this manner will hereafter be referred to as the servo clearance. Measurement of the glomerular filtration rate (creatinine and/or inulin clearance) in this manner eliminates the previously discussed inaccuracies of the classical urinary clearance measurement. In addition, this system allows continuous and nearly instantaneous calculation of the clearance and is thus appropriate for application to the study of factors which rapidly change the glomerular filtration rate.

In order to apply this method accurately, the tissues must be saturated with the substance under study, so that there is no net movement of that compound into these extravascular spaces. Greenburg et al. (65), after giving a priming injection followed by a constant infusion of creatinine to anesthetized dogs, found that the $[Cr]_p$ did not change after 85 minutes and they interpreted this to imply that an equilibrium had been attained within that time. Schloerb (139) found that in nephrectomized dogs, 100% of the final volume of distribution for creatinine was attained three to four hours after the start of a 30 minute infusion of a concentrated creatinine solution. Similarly, Williams et al. (171) observed that in nephrectomized dogs, the $[Cr]_p$ was constant 4½ to 6 hours after the start of a 30 minute infusion of a concentrated creatinine solution. After a single injection of creatinine in anuric patients, equilibrium was reached within 6 hours (44).

When inulin is constantly infused, equilibrium is reached after 60 minutes in unanesthetized men (41) and after two hours in unanesthetized dogs (60). Gotlove (33) found that in rats constantly infused with inulin, an initial rapid equilibrium was attained in one to two hours. A slower phase, accounting for about 10% of the volume of distribution, took up to 15 hours to reach equilibrium.

B.Discussion of Volume of Distribution

The volume of distribution for creatinine and inulin was also measured during these experiments. Dominguez (35) has said that, "The volume of distribution is the volume of body fluids which holds the substance in solution at the same concentration as the plasma." The concept of volume of distribution for a substance is deceptive, however, for the quantity measured in the calculation does not represent the volume in which the substance is distributed. As defined above, the volume of distribution does not represent any anatomical or physical space in the body, but merely represents a volume of fluid with concentration equal to that at the plasma. It is an equivalent rather than real volume. To illustrate this discrepancy, if a large quantity of iodide were given to a patient, it would become concentrated in the thyroid gland and the plasma concentration at equilibrium would be relatively low. If the volume of distribution were calculated on the basis of the amount injected and the equilibrium plasma iodide concentration, it would be enormous, whereas the iodide was actually distributed in the relatively small volume of the thyroid gland. Similarly, if a substance were distributed into a volume at a steady state concentration less than that of the plasma (e.g. Na^+ inside cells), the total magnitude of that volume would not be included in the volume of distribution calculation. It may be concluded that, unless all of the anatomically separate volumes have the same concentration for the substance as the plasma at equilibrium, the magnitude of these "volumes" is not correctly estimated in the volume of distribution calculation. The imprecision of this determination must be constantly kept in mind when one is interpreting the results of volume of distribution calculations.

This is especially true if any attempt is made to identify the anatomical space represented by a calculated volume of distribution.

The actual measurement of the volume of distribution for many substances is made difficult by the long equilibration times required for many of them (18,23,95,159). Cotlove has stated (33), "The principle factors delaying equilibration are : long radial diffusion distance, retarded diffusion through the capillary wall and interstitial matrix, small diffusion coefficient for the molecule and low blood flow relative to the volume supplies." Indeed, it has been suggested (33,138) that a distribution equilibrium in the thermodynamic sense is seldom attained in the body. The term "steady state" seems to be more appropriate when one is referring to the final dispersion of a substance within the body. Moreover, like most other biological parameters, the individual volumes within the body tend to fluctuate during the course of the measurement (18,23,172). This leads to difficulties in measurement and interpretation of the volume of distribution.

Thinking about the distribution of creatinine in the body is also complicated by the fact that plasma is not a thermodynamically ideal solution. Plasma is about 95% water and various investigators (44,139,141) have preferred to express the creatinine concentrations is terms of plasma water rather than plasma volume, and thereby calculate the volume of body water in which the creatinine is distributed. This is only partially correct, because it has been shown (59,78,98) that about 2% of plasma water is bound and not available to act as a solvent. The fact that creatinine is also bound to protein to a small extent (44,85,86) complicates the volume of distribution calculations. It seems, however, that if one is interested in the volume of body

fluid rather than body water (active or inactive as a solvent), this is best calculated by using the concentration of creatinine in the plasma for calculating the volume of distribution. The value calculated in this manner has solvent properties similar to that of plasma. The plasma creatinine concnetrations were used for calculating the volume of distribution in the present study.

The basic relationship, used in most volume of distribution calculations, is the dilution principle. This simply states that the volume of distribution is equal to the quantity of substance in the body divided by the plasma concentration. This relationship was used as early as 1915 by Kieth et al. (88) to measure the volume of plasma, and has found great application since isotopes became readily available (75). In nephrectomized dogs, Williams (171) measured the equilibrium $[Cr]_p$ after a 30 minute infusion of creatinine and calculated the volume of distribution to be 62% of the body weight after correcting for endogenous creatinine production. Using a similar protocol, Schloerb (137) found the creatinine volume of distribution to be 58% of body weight. In anuric patients, Edwards (44) injected a measured amount of creatinine and measured the equilibrium $[Cr]_p$ and calculated the volume of distribution to be 62% of body weight. A theoretical analysis of the $[Cr]_p$ curve after a single injection of creatinine into intact dogs resulted in a calculated creatinine volume of distribution of 76% of body weight (36) and 63% of body weight (35) by Dominguez. Sapirstein (138) analyzed the same type of curve in terms of a two compartment model and calculated the volume of distribution to be 36.8% of body weight. This technique, however, appears to be based on some incorrect assumptions concerning the distribution of the creatinine. Measuring the equilibrium

$[Cr]_p$ after a constant infusion of creatinine and assuming quantitative recovery of creatinine in the urine, Greenburg (65) found the creatinine volume of distribution to be 48% of body weight for unanesthetized dogs. Other investigators (7,91), however, have shown that creatinine is not quantitatively recovered in the urine.

On the basis of urine collections after equilibrium had been attained in dogs, the volume of distribution of inulin was calculated to be 15.7% of body weight by Schwartz (141) and 19.4% of body weight by Guadino (60). After a long equilibration time, Nichols (118) reports the inulin volume of distribution to be 22% of body weight. Values of 14.5% and 16.8% of body weight are reported for the inulin volume of distribution by Dominguez (35) and Swan (159) respectively. The inulin space in dog jejunum was found to be 39% by weight (4).

The volume of distribution of substances in the body has been the subject of many excellent reviews (35,42,43,60,99) and the reader is referred to these for a more thorough discussion of various techniques of measurement.

C. Results

In the present study, the creatinine clearance, computed on the basis of infusion data, approached, but infrequently equalled, the clearance calculated from the urinary data. It is to be recalled, that the servo clearance is the sum of the renal and the extra-renal clearances. The extra-renal clearance is large at the beginning of the experiment, due to the fact that creatinine is being pumped into the tissues at a high rate. As these tissue stores become saturated (i.e.

$\frac{dQ_{tissues}}{dt} \rightarrow 0$), the servo clearance assymptotically approaches the urinary clearance. Only when equilibrium is reached in the dog will the servo clearance equal the urinary clearance. In five of the animals (3064,1674,2374,1384,2084) an apparent steady state was reached, so that the rate of infusion of creatinine was a measure of the rate of renal excretion of that substance. Even in these animals, however, the infusion clearance was comparable to the urinary clearance only toward the end of the experiment. Reference to Table I will facilitate the comparison of the urinary and infusion clearances for individual experiments.

An apparent steady state was reached in the experiment on dog 1674 after 130 minutes, and this was maintained until 200 minutes. Over this interval, the average urinary creatinine clearance was 35ml./min. with a standard deviation of 11.4ml./min. For this same period, the average servo clearance was 36ml./min. with a standard deviation of only 6.7ml./min. The greater variance of the urinary clearance could be due to the fact that the bladder was apparently not completely emptied at 130 minutes, resulting in a false low value of that clearance and a false high value of the clearance at 150 minutes when the bladder residual volume was removed. This suggestion is supported by the observation that the urine volume collected at 150 minutes was 30ml. greater than that collected at 130 minutes. It appears, therefore, that when an apparent equilibrium has been reached, the infusion method can give a more consistent estimation for the renal clearance than the urinary collection method, which is subject to technical error.

Much the same result is evident in the experiment on dog 2374 in which a steady state for creatinine distribution was apparently

reached after 110 minutes and maintained for 50 minutes. The data from this dog are presented in the top panel of Figure 6, where it appears that the $[Cr]_p$ was well controlled and that the amount of creatinine accumulated in the dog was almost constant over the interval of 100 to 160 minutes. A graphic comparison of the servo clearance and the urinary clearance is presented in Figure 10. The average urinary creatinine clearance during this interval was 82.4ml./min. with a standard deviation of 7.3ml./min. Again, it is suggested that the variation in the clearance was due largely to technical diffulties with urine collection. These problems are obviously avoided by using the infusion data. This is reflected by an average servo clearance of 82.8ml./min. with a standard deviation of only 2ml./min. for the interval from 110 to 160 minutes in this experiment.

Figure 11 presents the comparison of the urinary and servo clearances for dog 1384. The clearance calculated from the infusion data is consistently higher than the urinary clearance until 150 minutes after the infusion has been initiated. This implies that an equilibrium has not been attained. The lower panel on Figure 6 shows that creatinine was accumulating in the dog's body during most of the experiment. It is interesting that in this animal the servo clearance during the latter part of this experiment does oscillate slightly. This parallels the oscillation in the $[Cr]_p$ during this period (Figure 6). It is thus concluded that when the oscillations in the $[Cr]_p$ are large, a significant error in the calculated servo clearance is introduced.

In dog 3084, as expected, the servo clearance is initially high and then falls gradually until approximately 110 minutes after the start of the experiment. At this time, the set point on the controller

was changed to maintain the $[Cr]_p$ at 10.0 rather than 8.5 mg.%. Before this change, however, the servo clearance approached the clearance measured from the urine data. The bladder of this dog was not washed with saline, however, and urine collections were therefore incomplete. This makes a meaningful comparison of the urinary and servo clearance difficult.

In dog 674, the servo controller was over damped, so the $[Cr]_p$ concentration continued to rise throughout most of the experiment. Therefore, the tissues never became saturated and no apparent equilibrium was attained, with the result that the servo clearance values were consistently higher than the urine clearance values. Similarly, in the experiment of dog 774, the servo controller was over damped and also the ureters were tied at 90 minutes, so equilibrium between the plasma and the tissues was not attained and the servo clearance was greater than the urine clearance. In experiment with dog 1374, equilibrium had not yet been attained by 100 minutes, at which time the controller set point was raised to maintain $[Cr]_p$ at 37 rather than 20 mg.%.

In dogs 1484 and 2084 only late in the experiment was an apparent equilibrium attained. This accounts for the servo clearance being consistently higher than the urinary clearance in these experiments.

Figure 9 presents a comparison of the servo clearance and the urinary clearance for inulin in three dogs. This compound reaches an apparent steady state after 30 to 60 minutes, as is evidenced by the nearly constant amount of inulin accumulated in the dog after this time. The initial high value for the inulin servo clearance is due to the rapid removal of inulin from the plasma into the other body fluid

spaces. The lower panels of this figure demonstrate that after 30 to 60 minutes, the servo and urinary clearances are nearly identical. In contrast to the results obtained with creatinine, it appears that the indirect servo method does allow an accurate estimate of the urinary clearance within a reasonable length of time.

Figures 10 and 11 demonstrate that the urinary creatinine and inulin clearances were very nearly identical during the experiments. This was typical of the results in all four of the animals in which these clearances were simultaneously measured. These results confirm the finding of previous studies (120,142,145) that the inulin and creatinine urinary clearances for dogs are equal.

The fluid balance in the dogs during the experiments is presented in Table II. The fluid volume in the animal was increased by the constant infusion of the solution which maintained the plasma concentration of the creatinine (inulin) at the desired level. The total volume of fluid infused depends upon the desired $[Cr]_p$, the creatinine concentration of the infusion solution, as well as the glomerular filtration rate. Blood was removed from each animal at the constant rate of 2ml./min. for the purpose of continuous measurement of the $[Cr]_p$. The small amount of blood removed for the purpose of standardizing the AutoAnalyzer has been ignored in the fluid balance calculations. The major source of fluid loss was by way of the urine, and Table II presents the total urinary volume for the experiment. The net deficit in the animals at the end of the experiment ranged from 0.5 to 8% of the body weight. Although this figure is not large, it is of sufficient magnitude to introduce a significant error in the calculated volume of distribution. For this reason, the value of 40.7% of body weight, which is the average

volume of distribution for creatinine in the five animals that did reach an apparent equilibrium, is of questionable physiological significance. It is to be stressed that this was a period of only apparent equilibrium which was characterized by an absence of accumulation of creatinine in the dog. From the data of other investigators which was presented earlier there are strong reasons for believing that if the experiments had been performed for longer periods, a larger volume of distribution would have been found. The difficulties in the determination of the volume of creatinine distribution in these animals are compounded by the fact that the circulation to the hind limbs was interrupted by arterial cannulae. The volume of distribution for creatinine will be dealt with in greater detail in the next chapter.

The average volume of distribution for inulin, in the four animals in which it was measured, was 16% of body weight. By subtracting the fluid deficit which existed at the end of the experiment from the weight of each dog, the average volume of distribution becomes 16.5% of body weight with a standard deviation of 4.2% of body weight.

D. Discussion

The validation of the servo clearance as a measure of renal function is difficult. The accepted standard for renal clearance is the value calculated on the basis of urinary data. By simply comparing the two clearances, it is not logically possible, however, to prove that the servo clearance is a more or less accurate measure of renal clearance than the urinary clearance. For this reason, the reproducibility of the clearance measurement by each method was used in order to assess the merit of each

clearance technique in the present study.

In the preceeding section, it was shown that in two experiments in which the servo and urinary creatinine clearances were approximately equal, there appeared to be less variation in the servo clearance than the urinary clearance. This is in accord with Merle et al. (41) who found that clearances measured by constant infusion of creatinine were more reproducible than those measured simultaneously by urine collections. The greater reproducibility of the servo clearance plus the practical and theoretical difficulties inherent in the measurement of renal clearande from urine data (e.g. need for quantitative urine collection and analysis, and dead time and dead space errors) suggest that, under certain conditions, the servo clearance is the preferable way to measure the renal creatinine clearance.

It is to be emphasized that this is true only under circumstances when the extra-renal clearance is zero. The results of the present study suggest that this is so about two hours after the elevation of $[Cr]_p$. This equilibration time is the major difficulty in the performance of renal creatinine clearance measurement by this method. This is the same difficulty encountered by Berger et al. (12) in their attempt to measure renal clearance of inulin and PAH by a constant infusion technique. The long time needed to attain equilibrium could be reduced by giving the animal an initial priming dose of creatinine. This has been used successfully by other investigators (65,89,91) in measuring the rates of excretion by constant infusion techniques. The use of a priming dose would allow the clearance measurement to be performed during a shorter interval and thus minimize the blood loss from the continuous sampling. With this modification, the technique could be applicable to clinical as well as

research investigations. The major disadvantage of using priming injections is that it is necessary to predict the volume of distribution and excretion rate in order to give the correct dose of creatinine to attain the desired $[Cr]_p$ (140).

It is realized that the urine clearance was not calculated in the classical manner in the present study. Rather than using a single $[Cr]_p$ measured at the midpoint of the urine collection period, the average $[Cr]_p$ as determined from the continuous $[Cr]_p$ record was used. As discussed in Chapter I, the errors introduced into the clearance calculation by using the $[Cr]_p$ determined at a single instant are significant, especially when the $[Cr]_p$ is fluctuating. It thus seemed more correct to use the average $[Cr]_p$ for the urinary clearance calculation. Using the same $[Cr]_p$ for both calculations also permitted a more direct comparison of the influence of the numerators in Equations 2 and 3 on the values calculated for the urinary and servo clearances respectively.

When compared to the creatinine clearance, the inulin clearance is more successfully measured by the servo technique. This is largely due to the fact that inulin apparently reaches a steady state in the dog, within 30 to 40 minutes after the $[In]_p$ is elevated to a constant level. After this time, the servo and urinary clearances are essentially identical. In view of the disadvantages discussed above, when compared to urinary clearance measurements, it seems that the servo method is the superior method for measuring the renal clearance of inulin. It is apparent that this technique would be improved if the $[In]_p$ were directly controlled by the servo mechanism. This is difficult, however, since there is not presently available a method for measuring $[In]_p$ at a rate that would permit direct control of the $[In]_p$ without wide oscillation

introduced by the analytical delay. It is for this reason that the $[In]_p$ was indirectly controlled by maintaining the $[Cr]_p$ constant. This was possible because the renal excretion of inulin and creatinine were similar in the present study. It is suggested that if a fast method for measuring inulin were found (perhaps by radioactive isotopic labelling), the servo clearance technique would be suitable for clinical application in the same way as the continuous infusion method (12).

Mannitol diuresis was maintained in these experiments in order to insure adequate urine volumes. This drug has been reported to increase the renal blood flow (5,17,64,109,115) by most investigators, with few exceptions (172). This increase has been attributed to increased blood volume (5), increased cardiac output (115), decreased blood viscosity (64,109), and a decreased renal vascular resistance (17, 64,109). It has been amply shown that mannitol does cause significant fluid shifts in the body that result in augmentation of the circulating volume (5,31, 114,115,176) and a diuresis that may result in secondary hemoconcentration (63). Mazze, Schwartz and Berry (100) have found, however, that "infusion of hypertonic mannitol alone or combined with prior water loading, did not significantly increase the clearance of PAH, inulin and creatinine in human subjects with or without renal disease."

Sodium pentobarbital anesthesia, as used in the present study, is routinely used in many physiological experiments and induces few changes in the function of the organism, aside from the anesthetic effect. A slight elevation of the plasma volume has been reported (160,161) as well as an elevation in cardiac output (161) and an increase in peripheral blood flow secondary to vasodilatation under autonomic nervous influence (82).

Since the animals were losing blood at the rate of 2ml./min, a decrease in the blood pressure was noted in spite of the creatinine-mannitol infusion. The fluid shifts associated with a drop in blood pressure have been previously recognized (114). The general decrease in tissue perfusion does prolong the time necessary for distribution of the indicator into the entire body (61). The kidney perfusion seems to be well preserved in spite of compromised blood volume and decreases only slightly as the blood pressure falls (145). It was noted (84,97), however, that the glomerular filtration rate did tend to fall toward the end of these experiments. This was presumably due to terminal shock. In accord with other investigators (84,142) it was found in these experiments that the inulin/creatinine clearance ratio remains close to unity during periods of relative tissue hypoxia.

The method of measuring the rate of removal of a substance from the body by measuring the rate that the substance must be infused in order to maintain a constant amount in the experimental subject is not limited to creatinine and inulin. It is to be expected that it could be used for any non-metabolized substance. It is suggested that the problems encountered in this study, such as the long time for equilibrium to occur and artifacts introduced by sampling techniques, should be kept in mind when applying this method to other compounds.

Although it was possible to calculate the volume of distribution for inulin and creatinine in this study, it is to be emphasized that these experiments were not designed specifically for this purpose. The fluid deficits that existed in these animals make the calculated volumes of distribution difficult to interpret. The volume of distribution for inulin which was calculated in this study agrees well with that deter-

mined previously (35,60,141,159) for comparable equilibration time. It has been suggested (4,60,118) that the inulin is distributed into the total extracellular fluid of the body which Edelman (42) describes as consisting of: a) plasma, b) interstitial and lymphatic fluid, c) fluid in dense connective tissue, cartilage and bone, and d) transcellular fluid. Estimates of the extracellular volume have ranged from 19.4% (99) to 27% of body weight. As previously stated, unless the inulin partition coefficient for all the subdivisions of the extracellular space is unity, the volume of distribution of inulin will not correctly measure the extracellular volume. Indeed, it has been shown (42) that only about 10% of the bone water is accessible to polysaccharide tracers. Even when an apparent equilibrium is reached in the body, the CSF inulin concentration is only 10% of that in the plasma (159). It appears, therefore, that the volume of distribution for inulin, which does not penetrate cells (4), is an underestimate of the volume of extracellular fluid.

In summary, this chapter has presented data which suggest that the servo clearance method, proposed in order to circumvent some of the limitations and errors inherent in the urinary clearance method, can correctly estimate the renal creatinine clearance only under certain conditions. The rate of creatinine infusion was a reflection of the rate of renal excretion after approximately two hours, when the tissue stores had reached an apparent steady state. After this time, the clearances calculated by Equations 2 and 3 were similar in some experiments and it was demonstrated that the servo clearance exhibited less variation than the urinary clearance in two animals. The disadvantage of the long equilibration time was not so severe in the determination of the inulin clearance by the servo method and the four experiments

in which $[In]_p$ was indirectly controlled demonstrate that the servo method can be both accurate and practical. In this chapter appears a discussion of the creatinine and inulin volumes of distribution and this quantity was calculated for each of the animals. In view of the unfavorable fluid balances which existed in these experiments, the significance of this calculation is questionable. The influence of mannitol, pentobarbitol and blood loss on the renal function of the animal has been reviewed. Modifications of the servo method are suggested in order to improve its practicality for further applications.

CHAPTER III

COMPUTER MODEL FOR THE DISTRIBUTION OF CREATININE IN THE DOG

A. Introduction

This chapter presents the computer solution of the equations in a mathematical model that describes the distribution of creatinine in the dog. Harmon and Lewis (71) have defined the term "model" as "that which is similar in function, but differs in structure and origin from that which is modelled." Berman and Mones (10) have restricted the term "to represent any set of equations or functions that describe the response of a system to a stimulus." In a broader sense, however, a physiological model can be considered as an outgrowth of man's attempt to describe complex living systems in terms of simple physical, chemical or mathematical systems with which he is more familiar (48). Ideally, a biological model is a coherent, concise, functional description of reality as it exists in nature. It must be stressed, however, that this is a simplified reflection of reality which is a limitation imposed by lack of data.

Aside from formalizing the thought on a particular topic, models serve many important functions in physiology. Models can be used to synthesize disparate data into a single consistent view (148). A useful model will raise new questions and facilitate the testing of suggested hypotheses. Prediction of new relationships and implications that will direct future research and experimentation is also a useful result of modelling. Especially if the model is constructed on a computer, simulated experiments may be performed more quickly and economically than is possible in actual biological tests.

In this chapter, two models are described which were used to

simulate the experiments described in Chapter I. The first is a mathematical model which describes the response characteristics of the creatinine sampling system. Both analog and digital computers were used to construct this model. These computers were programmed so as to produce the same delay and distortion in a step input of voltage that was actually observed when the sampling system responded to a bolus of creatinine solution. The second model was a compartmental representation of the experimental subject and is therefore of greater physiological interest. An analog computer was used to construct this model. Various formulations of the animal model were proposed and tested on the computer and a single model was formulated which was capable of simulating the experimentally observed responses of the dog. The animal and sampling system computer models were used simultaneously in order to simulate the experiments described in Chapter I. In order to evaluate the implications, limitations and validity of this simulation, it is first necessary to discuss some of the general principles of compartmental analysis as applied to this problem.

B. Assumptions and Definitions

A kinetic analysis of the distribution of any substance in the body must be based on certain simplifying assumptions and definitions. The usual approach, as outlined in a number of reviews of this technique (16,42,43,92,132,151), is to divide the total volume of distribution for a particular substance into subunits which are either anatomically or functionally discrete. These subunits, or compartments, have been defined by Robertson (131) "as regions or volumes in which a uniform con-

centration of the substance is assumed at all times." "In a practical sense, an instantaneously mixed compartment may be assumed whenever the turnover time of compound in that compartment is long, relative to the time required for mixing in the compartment" (154). In this study, a compartment is defined as the mathematical representation of a body space in which a substance can be considered to be instantaneously mixed and of uniform concentration.

It is to be noted that this definition is expressed in functional rather than anatomical terms. Cotlove (33) demonstrated that "the process of distribution of substances in extracellular fluid involves multiple rate components which do not reflect fixed anatomically separated compartments." Other investigators (42,118,132,169) have also found that a single, clearly defined, anatomical space in the body actually responded in tracer experiments as if it were composed of a number of compartments with quite different volumes and permeabilities. On the other hand, some investigators (18,23,36,138,151) have found that the analysis of kinetic data has required that various anatomical compartments be combined to form a single functional component. Solomon (156), when discussing the identity of the functional compartments that were derived in his kinetic analysis, has stated that "One does not say that the compartments B and C exist as such in the body, but rather that one, say B, represents the average of a number of areas to which sodium is transferred quickly and the other, C, the average of a number of areas to which the sodium is transferred more slowly." Sharney (145,146) has discussed the mathematical implications of combining individual compartments to form the larger compartments that are detected in tracer experiments. The fact that the functional compartments derived from a kinetic analysis can not always be

anatomically identified has been emphasized by Zierler (174). Shore (154) pointed out that if the experimental data can be fitted by a compartmental model, then the experimental data can be equally well explained by a model composed of a larger number of compartments. It is therefore not logically correct to assume that a model composed of a given number of compartments necessarily represents the same number of anatomical compartments. Even if the individual compartments in tracer experiments could be anatomically identified, however, the validity and usefulness of speaking in terms of the creatinine concentration in a particular tissue of fluid space must be questioned. Every organ is composed of a number of subunits in different physical phases. This was emphasized by Garfinkel (56) who suggested that there are functionably detectable compartments at the sub-cellular level. This must be kept in mind when considering the functional significance of the creatinine concentration in a particular organ or fluid space. In summary, the present study is concerned with functional rather than anatomical compartments, for the following reasons: a) seemingly homogeneous, anatomical compartments behave as if they were composed of functional subunits, b) anatomically separate compartments may react similarly, and c) the division of body compartments along anatomical boundaries is sometimes of questionable functional signigicance. It is realized that the study of functional rather than anatomical compartments does not fulfill all of the criteria of an ideal model mentioned above, but this shortcoming is due to lack of appropriate data that would allow a definite anatomical study of the distribution of creatinine.

The volumes of the compartments were assumed to be constant in this analysis. Only the amount of creatinine in each compartment was

considered to change during the experiment. Since data from only the beginning of each experiment was used, the difference between the fluid infused and excreted was not significant (see Table III). The average fluid deficit was about 0.5% of body weight. It is understood that fluid shifts did occur within the body of the animal (see mannitol, pentobarbital and hemorrhage effects in Chapter II), but with the present data it is impossible to correct for these entirely. By using the data from the initial 60 to 90 minutes of each experiment, however, these effects can be expected to be minimized, thereby validating the assumption that the compartmental volumes are constant.

The third assumption that was made in this analysis is that the creatinine in each compartment is distributed uniformly. As suggested previously (18,23) in order for this assumption to be valid, it is not necessary that the tracer be mixed instantaneously in the compartment, but rather that the time for mixing be short, relative to the rate of turnover of creatinine in the compartment. This assumption also implies that the various components of any compartment are equally permeable to the creatinine (154) and that the partition coefficients are constant throughout the experiment (105).

It is also assumed that the amount of creatinine synthesized and metabolized by the animal during the simulation is negligible. The validity of this assumption is supported by the work of many investigators (44,65,79,91,139) presented in the Introduction. The amount of creatinine removed by the arterial blood sampling has been neglected in this analysis, because it was less than 3% of the amount of creatinine lost from the animal during the period of the simulation.

It is further assumed that the elevation of the creatinine con-

tent of the animal does not disturb the manner in which the creatinine is handled in the body. The supposition that the infused and endogenous tracer behave similarly is fundamental to all compartmental tracer analysis (23,156). In the present case, the assumption is supported by the demonstration (37,79,108) that even at elevated concentrations, the rate of creatinine excretion is proportional to the plasma creatinine concentration, which is the same manner in which creatinine is excreted at normal concnetrations (38,62,116,164).

It is also assumed in this study that the initial concentration of creatinine in each compartment was equal to the endogenous $[Cr]_p$. In the two other creatinine distribution models, the initial creatinine concentrations in the peripheral compartments were neglected entirely (35,36,138). Although no data are available for the creatinine content of various canine tissues, Baker et al. (1) found that the creatinine concentration in rat muscle was about three times larger than $[Cr]_p$, while the creatinine concentration is parenchymal organs ranged from 10% to 50% of the $[Cr]_p$. The intracellular creatinine concnetrations were closely correllated with the creatine content (1), which is the precursor for creatinine (73). This suggests that the cell wall is a barrier for the diffusion of creatinine (see below). Mc Gaughey et al. (110) report that the creatinine content of human amnionic fluid is about three times that of the plasma. In rabbits, Bradburg and Davson (21) discovered that the creatinine concentrations in the cerebrospinal fluid and brain were about one third as high as $[Cr]_p$. In the present analysis, the intracellular water space was represented as a single compartment (see Derivation of the Creatinine Distribution Model). Shore et al. (154) have said "the amount of compound calculated to be present in the lumped

tissue compartment will approximate the sum of the amounts of compound in each of the individual compartments." Since the creatinine concentration in some tissues is more than $\left[Cr\right]_p$ and in some tissues it is less, it was therefore considered reasonable, though not necessarily correct, to assume, on the basis of the information presently available, that the average initial tissue concentration was equivalent to the endogenous $\left[Cr\right]_p$.

It was further assumed that the amount of creatinine transferred between two contiguous compartments was directly proportional to the creatinine concentration gradient between them. The constant of proportionality, called the rate constant, is characteristic of the route of exchange and was assumed to be constant. This supposition concerned with the transfer of creatinine describes the exchange of this substance between the components of the circulation. If creatinine were to move between the compartments by the process of diffusion, the rate of transfer would be proportional to the concentration gradient. Renkin (129) has stated that "diffusion is the process responsible for nearly all exchange of metabolic substrates and products between blood and tissues." The validity of this statement has been proven for a number of compounds (21,33,128). Van Slyke et al. (165) and later Gary-Bobo et al. (58) have demonstrated that the creatinine penetration into erythrocytes is a diffusion process. Similarly, Bradburg and Davson (21) found that creatinine diffused between the cerebrospinal fluid and the plasma. In a series of in vitro experiments, Mc Gaughey et al. (110) demonstrated that the transport of creatinine across an isolated chorio-amnionic membrane occurs solely by diffusion, rather than be active transport.

It was also assumed that the rate constants for influx and eflux

along the same route were equal. Solomon (156) has emphasized that this is a necessary corollary, when the mechanism of transfer between the compartments is diffusion.

The time delays for the transport of creatinine in the dog's body have not been directly incorporated into the present model. This assumption is commonly made when constructing models for the distribution of diffusible indicators in the body (15,35,36,51,56,61,66,105,128,130,138, 154,166). The circulation of the creatinine in the plasma is one of the major sources of this delay. This must be considered when studying the dispersion of an indicator in the circulation (8) because these delays significantly alter the shape of the observed dye curve. These delays are short, however, when compared to the relatively long time involved in the distribution of creatinine throughout the body. The inevitable time delays were partially accounted for, however, by constructing the model as a series of compartments from which the creatinine moved in an exponential manner (147).

Finally, it was assumed that the urine excretion occurred from the compartment analogous to the arterial plasma. The urine excretion was also assumed to be equal to the average urinary clearance measured during the period of the simulation (36). Since creatinine is removed by glomerular flitration in the dog (120,142,145), it is justified to assume that the rate of removal of creatinine is directly proportional to the arterial $\left[Cr\right]_p$. Indeed, Dominguez and Pomerene (37) have shown that the relationship between the plasma creatinine concentration and urinary creatinine excretion is linear.

The above assumptions have been the basis of many compartmental kinetic analyses for a variety of compounds distributed in the body (2,

35,88,144,149, for example). Stephanson and Jones (158) have shown that the experimental assumptions that are customarily made in compartmental analysis can be interpreted directly in terms of the axioms which characterize a linear vector space and a linear operator. These assumptions form a special case of a general theory proposed by Kerner (90) dealing with the treatment of kinetics in chemistry and physics. In the present model, the analog of the animal was constructed from differential equations, but other investigators have approached similar analyses using integral equations (22), convolution theory (150,175) and La Place transforms (152).

C. Derivation of the Creatinine Distribution Model

The models for the distribution of creatinine in the dog that were previously proposed have been based on assumptions similar to those presented above. Dominguez et al. (35,36) suggested a model consisting of a compartment, into which creatinine is infused and excreted, which exchanges creatinine with a second compartment at a rate proportional to the concentration gradient existing between the two. The first compartment was identified with the extracellular fluid space while the second compartment corresponded to the intracellular water. The shape of the $[Cr]_p$ curve following the injection of a single bolus of creatinine was analyzed in terms of this model and the rate constants and compartmental volumes were calculated. It was noted, however, that the initial part of the curve, which corresponds to the mixture of the creatinine in the animal, could not be accounted for by this model.. This discrepancy is perhaps due to the assumption that the creatinine became homogeneously

distributed throughout its entire volume of distribution; this assumption was questioned by Sapirstien et al. (138) who emphasized that equal plasma and extravascular creatinine concentrations exist only momentarily under these conditions.

Sapirstien et al. (138) therefore proposed a model based on the assumption that creatinine is distributed between two compartments and moved between the first and second in proportion to the concentration difference between them, while being excreted from the first in proportion to its concnetration there. They found that the $[Cr]_p$ disappearance curve after a single injection of creatinine could be resolved into the sum of two exponential curves in seven of ten dogs. Again, the early part of the curve was neglected. There was, however, a wide variation in the compartmental volumes and rate constants for various experiments. This implies that the model was not unique (16,167). Moreover, in this model, it was incorrectly assumed that the tissue creatinine concentration was initially zero. Robertson (177) presents a four compartment catenary model which correctly computes the blood creatinine concentration during and immediately after a 30 minute infusion of creatinine. However, the details, such as compartmental volumes and intercompartmental rate constants for the model, are not given by the author.

The exact form of a compartmental model is dependent upon the data available to test the model as well as the phenomenon being modelled. In the present analysis, the rate of creatinine infusion into the animal, the time course of the arterial $[Cr]_p$, and the rate of urine excretion were measured. It is self evident that the dynamics of a model for the distribution of creatinine in the dog are more rigorously tested by the continuous infusion of creatinine. As pointed out by Sheppard and House-

holder (151), however, the formulation of a model on the basis of plasma concentration data alone is difficult, because the plasma concentration can be quite insensitive to the change in the concentration in the extravascular compartments. It is with this limitation in mind that the following model was formulated.

It is suggested, on the basis of evidence presented in Chapter II and on that of other investigators (35,36,44,65,138,139,171), that the creatinine volume of distribution is of sufficient magnitude to indicate that creatinine is distributed into the plasma, extracellular and intracellular fluid volumes. This suggestion is supported by the fact that creatinine distribution in the body is mainly by diffusion rather than by an active transport mechanism, which implies that it is not actively concentrated in a particular space.

In view of these considerations, the model for the distribution of creatinine in the dog was initially constructed of three compartments, connected in series, with infusion into and excretion from the first compartment. These three compartments were roughly equivalent to the plasma, interstitial fluid and intracellular fluid. The division into three compartments seemed justified as a first approximation, because it has been shown that both capillary walls (81,93,110,128,129,152) and cell membranes (76,85,86,165) are barriers to the passage of diffusible indicators. The exact volumes of these compartments were not known initially, because, as discussed previously, they tend to vary with the physiological condition of the animal.

The plasma volume for dogs has been found to be approximately 4.5% of body weight by a number of investigators (35,41,135,155). The total functional interstitial fluid volume is approximately 16% of body

weight as determined by indicator dilution technique (35,99,118,159). The total body water determined by both desiccation and indicator dilution methods is approximately 63% of body weight (35,75,99,139,171). After pointing out that some components of the body fluids, expecially that in bone and dense connective tissues, are not penetrated by diffusible indicators, Edelman (42) has estimated that the functionally accessible intracellular fluid volume is approximately 33% of body weight. The analysis in the present case was additionally complicated by the interruption of the circulation to the hind limbs by arterial cannulae.

In practice, it was found that, when this model was programmed on the computer with the observed rate of creatinine infusion used as the driving function, a three compartmental model was not adequate to account for the variation in $[Cr]_p$ observed in our experiments. The three compartmental model was not sufficiently flexible to achieve a good fit between the observed and computed curves. It was necessary to divide the compartment corresponding to the plasma into two compartments. The first compartment was analogous to the plasma volume into which the creatinine was directly infused, in addition to the rapidly perfused segment of the circulation. This compartment, therefore, included the central venous reservoir, the plasma volume in the heart and lungs, in addition to the arterial plasma volume and the rapidly perfused capillary beds, such as the kidney and heart (137). This is in accord with the finding of Chinard et al. (28) that the distribution of creatinine in the isolated kidney is flow rather than diffusion limited. The second compartment corresponded to the volume of vascular fluid contained in the capillaries and the veins returning to the vena cava. The first and second compartments are functionally discrete, because the flow in the

arterial system is much swifter than the flow in the capillary and venous systems. The initial compartments also include that small fraction of the extravascular fluid which Edelman (42) states is rapidly exchanging with the plasma. Serial photomicrographs confirm that the immediate pericapillary fluid space is rapidly penetrated by diffusible indicators (81). It is interesting that Gellhorn et al. (61), when studying the rate of transcapillary exchange of sodium in the dog, found that this electrolyte was rapidly distributed into a volume approximately twice as large as the known plasma volume. Since this exchange occurs at the capillary level, this small contribution from the extravascular fluid forms a larger fraction of the second compartment (which includes all of the peripheral, slowly perfused capillaries) than the first compartment (which includes only the pulmonary capillary bed). The pulmonary extracellular and intracellular fluid spaces should ideally be included as a series of two additional compartments arising from the first compartment, but these volumes are so small when compared to the total body water (99) that they may be lumped into the other peripheral compartments without introducing a significant error in the simulation. The volumes of the peripheral compartments were considered as variables which could be adjusted so as to achieve the best fit between the observed and computed curves. However, the restriction placed upon this variation was that the volume of the fourth compartment, which represented the intracellular compartment, was approximately one half of the total volume of distribution, as suggested by Edelman (42).

The rate constants for exchange between the compartments were not initially known with much accuracy and were considered as parameters which could be adjusted to achieve a satisfactory simulation. The effect

of adjustment of the rate constant is inversely proportional to the variation of the compartmental volumes. This method of model refinement was suggested by Berman (15) and applied by Fleischli (51) in his study of the distribution of d-tubocurarine and by Robertson and Cohn (130) in the analysis of calcium and strontium metabolism. The rate constant for the exchange between the first two compartments is an approximation of the cardiac output of the animal, and the cardiac output for the normal dog reported by Sapirstein (137) was used as a first approximation for the value of this rate constant. Since the cardiac output for the animal is subject to side variation, this rate constant was adjusted to refine the simulation. It was one of the objectives of this simulation to determine the magnitude of the rate constants for the exchange in the peripheral compartments. Figure 14 presents the four compartment catenary model used in the present simulation.

D. Derivation of Equations for Model of Distribution in the Animal

At any time (t), a compartment i is characterized by a volume V_i, a creatinine concentration $c_i(t)$ and a total amount of creatinine contained in the compartment $q_i(t) = c_i(t)V_i$. Since the rate of movement of creatinine from a compartment is directly proportional to the creatinine concentration in that compartment, the movement of creatinine along a particular route j may be represented as $\frac{dq}{dt} = K_j c_i(t)$. The rate constant, K_j, is characteristic of the route of exchange. Thus, the model consisting of four compartments connected in series with infusion $\frac{dq}{dt}$ into and excretion $K_u c_1(t)$ from the first compartment (Figure 14)

is described by the following eqations:

$$V_1\frac{dc_1}{dt} = \frac{dq}{dt} + K_1(c_2 - c_1) - K_u c_1 \quad (3)$$

$$V_2\frac{dc_2}{dt} = K_1(c_1 - c_2) + K_2(c_3 - c_2) \quad (4)$$

$$V_3\frac{dc_3}{dt} = K_2(c_2 - c_3) + K_3(c_4 - c_3) \quad (5)$$

$$V_4\frac{dc_4}{dt} = K_3(c_3 - c_4) \quad (6)$$

In order to solve the above equations for the individual compartmental concentrations, it is necessary to integrate Equations (3) - (6). For this purpose an analog computer was used. It was therefore necessary to express the real variables (c, q, t) in terms of computer varibles (C, Q, T). The following scaling equations were used for this purpose:

$$C \text{ volts} = X_c \frac{\text{mg volts}}{\text{ml}} \quad c \frac{\text{mg}}{\text{ml}} \quad (7)$$

$$Q \text{ volts} = X_q \frac{\text{volts}}{\text{mg}} \quad q \text{ mg} \quad (8)$$

$$T \text{ computer sec} = X_t \frac{\text{computer sec}}{\text{real sec}} \quad t \text{ real sec} \quad (9)$$

Here X_c, X_q, X_t are scaling constants. The values used in the experimental simulations appear in Table V.

Equations (3) - (6) scaled for the analog computer follow:

$$V_1\frac{dC_1}{dT} = \frac{X_c}{X_q}\frac{dQ}{dT} + \frac{X_1}{X_t}(C_2 - C_1) - \frac{K_u}{X_t}C_1 \quad (10)$$

$$V_2\frac{dC_2}{dT} = \frac{K_1}{X_t}(C_1 - C_2) + \frac{K_2}{X_t}(C_3 - C_2) \quad (11)$$

$$V_3\frac{dC_3}{dt} = \frac{K_2}{X_t}(C_2 - C_3) + \frac{K_3}{X_t}(C_4 - C_3) \quad (12)$$

$$V_4\frac{dC_4}{dt} = \frac{K_3}{X_t}(C_3 - C_4) \quad (13)$$

Equations (10) - (13) formed the basis for the analog computer program used to simulate the experiments described in Chapter I.

E. Sampling System Model and Technique of Simulation

Both analog and digital computers were used to solve the complex differential equations which describe the model for the distribution of creatinine in the dog. Since Chance (24) first used an electronic differential equation solver in the study of biochemical reaction kinetics, computers have been applied with great success to a variety of physiological research problems. The advantages of using computers in model construction include: (a) high speed in calculating the solution of the equations, (b) the ability to solve difficult problems, and (c) the ease of application of non-linear functions to biological models (71,122).

Like Chance, other investigators have used computers based on simple R-C circuits (77,105). The introduction of the analog computer, which is a collection of high gain amplifiers with either resistance or capacitance feedback, has facilitated the solution of differential equations (51,130,167). Many reviews of the general properties of analog computers are available (25,66,124, for example).

The digital computer has also been used extensively to solve the differential equations forming biological models (25,117,124,154,166). Many general programs for fitting the parameters of compartmental models to the observed data have been proposed for the digital computer (15,16,56,66). When compared to the analog computer, the digital machine provides the advantage of flexibility. It can therefore be used to

generate complex functions (157) and simulate pure delays, both of which are useful in the simulation of biological experiments (49). The hybrid computer, which is the combination of digital and analog computing elements used simultaneously, is particularly suited for the kinetic analysis of the distribution of substances in the body (102). A hybrid computer system was used in the present study. Before discussing the computer method for simulating creatinine distribution in the dog, the simulation of the sampling system will be presented.

The sources of the dispersion and delay introduced by the Auto-Analyzer system are discussed in Chapter I. For the purpose of this simulation, the curve in Figure 4 was copied into an electronic function generator, where it was approximated by ten straight line segments of variable slope and length and the angles of intersection of the line segments were smoothed into curves. A ramp of -50 to +50 volts was used as the abscissa for this function generation (Figure 13).

The distortion introduced by the sampling system was simulated on the analog computer by constructing a fourth-order Paynter filter (67). The equation and analog computer circuit for this filter is given in Figure 12. It was found that a filter with a frequency w_o 70 radions/sec. distorted a voltage step input to approximately the same extent as the sampling system distorted a creatinine step input (Figure 13).

The fourth-order Paynter only accounted for part of the time delay introduced by the sampling system. The remainder of the delay was simulated by converting the voltage of the distorted sample into a digital form and using this digitalized data as input to a CDC 3200 digital computer which was programmed * to store these data for a pre-selected

* I am indebted to Dr. J.B. Bassingthwaighte for writing the program for the CDC 3200 computer.

time and then transmit the data to a digital to analog converter from which the voltages were transmitted to the analog computer (Figure 12). The storage time in the digital computer was adjusted to achieve a good fit with the observed delay (Figure 13). The effect of the simulated sampling system is also demonstrated in Figure 18, where the creatinine concentration before (C_1) and after (C_{sim}) passing through the simulated sampling system is presented.

The simulation of the animal experiments was carried out by constructing an electrical analog model which responds to simulated creatinine infusion in the same manner as the animal responded to the experimental creatinine infusion. Figure 16 presents the general method of the present simulation. To accomplish this, from the continuous records of the $\left[Cr\right]_p$ and total creatinine infused into the animals used in the experiments described in Chapter I, readings were made at 20 second intervals. These values were entered into punched cards. A program was written for the IBM 7040 which used these data to compute the rate of creatinine infusion, $\frac{dq}{dt}\frac{mg}{sec}$. Under the direction of a second program, the IBM 7040 read the digital value of the rate of infusion and the plasma creatinine concentration and also generated a square wave pulse which was required to trigger the integration of the analog computer. These data arrays were emitted repeatedly by the IBM 7040 to be translated into voltages by an SDS D/A converter. These three voltages were transmitted to an Ampex 14-channel tape recorder, where they were continuously recorded on three separate channels for permanent storage. The recording on the analog tape minimized the computer time needed to generate the functions.

The recorded triggering pulse preceeded the appearance of the

infusion and concentration arrays by a few seconds. Each time that the triggering pulse was emitted from the tape recorder, it was amplified twenty times and then used to initiate an electrical ramp of variable slope in a saw-tooth wave generator. When the ramp voltage exceeded a selected value, a pulse, initiated from a pulse generator, discharged the relays which initiated the sweep of the oscilloscope and the integration by the analog computer (Philbrick Researches, Inc., Boston, Mass.). The slope of the electrical ramp was adjusted so that the analog computer began its integration at precisely the moment that the voltage representing the rate of creatinine infusion was transmitted to the analog computer from the tape recorder. After the recorded voltage representing the creatinine concentration had been transmitted to the analog computer, it was amplified and scaled appropriately and then displayed on the 8-channel oscilloscope. Similarly, the voltage representing the rate of creatinine infusion was amplified, scaled and used as an input driving function for the electrical analog of the dog.

The analog computer model for the distribution of creatinine in the dog is presented in Figure 13. It consists of four integrators, one representing each of the four compartments in the model. The scaled voltage representing the rate of infusion is one of the inputs to the first integrator. The initial condition for each of the integrators was the appropriately scaled endogenous plasma creatinine concentration, which was measured at the beginning of the experiment. The mathematical expression represented by each of the 11 potentiometers A through K is presented in Talbe IV.

The voltage in the analog computer which represented the simulated arterial $\left[Cr\right]_p$ was then transmitted to the model of the AutoAnalyzer

sampling system. After emerging from the simulated sampling system, this voltage, $C_{sim}(T)$, was visually compared on the oscilloscope with the scaled voltage representing the observed AutoAnalyzer record. The parameters of the analog model of the dog were adjusted, within the limitations mentioned previously, to attain the best possible fit of the observed and simulated arterial plasma creatinine concentrations. Photographic records were made of the oscilloscopic tracings representing these concentrations, as well as the creatinine concentrations in the various compartments (see Figures 17, 18, 19).

A record of the total amount of creatinine infused was made by electrically integrating the voltage representing the rate of creatinine infusion, $\frac{dQ}{dt}$. The quantity of creatinine excreted was computed by integrating the product of K_u and the voltage representing the concentration of creatinine in the first compartment (C_1). The amount of creatinine accumulated in the body of the dog was computed by electrically subtracting the voltage representing the quantity excreted from the amount of creatinine infused. Photographic records of these three curves were also made (Figures 17, 18, 19). Figure 20 is a photograph of the analog computer as programmed for the present simulation.

Five of the nine animal experiments were selected for the simulation. The experiments that were selected had a relatively wide fluctuation in the plasma creatinine concentration. It was felt that these experiments provided the most severe test of the analog model. In the animal experiments that were not selected for simulation, the plasma creatinine concentration changed very little, so these experiments were not a rigorous test for the analog model.

F. Results

Reference to Figure 13 demonstrates that the hybrid computer model of the sampling system closely simulated the characteristics of the experimental sampling system response to a step input of creatinine. As a component of the entire simulation, the most important contribution of the sampling system analog was the delay that it produced. This is seen in Figure 18, which demonstrates that the wave form of the output of the sampling system analog is quite similar to the input, and that it differs mainly in time delay. Had the oscillations of the plasma creatinine concentration been of a higher frequency, however, the amplitude of the oscillation would have been reduced by the sampling system analog.

In the five animal experiments that were simulated, satisfactory agreement between the computed and the observed arterial plasma creatinine concentrations was obtained with the analog model presented in Figure 14 and Figure 15. Table IV presents the numerical value of the analog computer potentiometers for each of the experiments, along with the mathematical expression represented by each potentiometer. By substituting the numerical values of the scaling constants presented in Table V into these expressions, it is possible to calculate the magnitude of the various rate constants and compartmental columes. The results of these calculations appear in Tables VI and VII.

Figure 17 presents the results of the computer simulation of the creatinine distribution in dog 2084. The top panel demonstrates that the computed and the observed plasma creatinine concentrations were almost identical. The concentration of creatinine in the first compartment closely resembles the simulated AutoAnalyzer tracing, except that the

curve is not delayed. The slight oscillations that are present in the creatinine concentration in the first compartment are diminished in amplitude in the second compartment and are decreased still further in third and fourth compartments. It is interesting to note that after approximately 30 minutes, the majority of the creatinine accumulating in the animal is flowing into the fourth compartment, which corresponds to the intracellular fluid space. Even after 90 minutes, the creatinine concentration in the distal compartments was not equal to that in the proximal compartments. This implies that the penetration of creatinine into the cells is the rate limiting step in the attainment of equilibrium for creatinine in the body.

The experiment in dog 1674 presented in Figure 18 is a severe test of the validity of the analog model because $\left[Cr\right]_p$ in this animal fluctuated widely. In this simulation a good agreement between the observed and computed arterial plasma concentration was obtained. The interesting aspect of this experiment is that the rate constant for exchange between the first two compartments is quite low when compared to the other experiments which were simulated. This could be interpreted to imply that there was a significant element of vasoconstriction in this particular animal.

The simulation of the distribution of creatinine in dog 3064 is reproduced in Figure 19, Here, once more, the observed and computed arterial plasma creatinine concentrations were nearly identical. By 90 minutes, the creatinine concnetration in all four compartments was nearly equal. This is in accord with the finding that the servo clearance and the urinary clearance were similar at this time (Table I).

In Tables VI and VII the average compartmental volumes and rate

for the five simulated experiments are presented. The average volume of the first compartment is 2.04% of the animal's body weight. This compartment includes the central venous reservoir, pulmonary plasma volume and the arterial plasma volume, in addition to that fraction of the capillary circulation which exchanges rapidly with these vascular spaces. Although there are no other reported measurements of this particular volume, this value does seem reasonable, in view of the fact that the plasma volume has been repeatedly found to be 4.5% of the body weight (35,42,135,155). The second compartment, with an average volume of 7.14% of the body weight includes the peripheral capillary beds and venous pools, in addition to that small fraction of the extravascular fluid which is rapidly exchanging with the plasma. The total volume of the first three compartments averaged 20.9% of body weight, which is quite close to the sum of the plasma and accessible interstitial fluid volumes quoted earlier. The fourth compartmental volume averaged 22% of the body weight. This is approximately two-thirds of the total intracellular fluid volume, as measured by other investigators cited above. This suggests that a significant fraction of the intracellular fluid space is not penetrated by creatinine. On the basis of the data from this analysis, the identity of this cellular space can not be definitely determined, but it is suggested that the devascularized segments of the hind limbs might be a significant portion of the unpenetrated cellular volume.

The average rate constant for the exchange between the first two compartments was 26.1ml./sec. Since this exchange represents the movement of the plasma creatinine between the arteries and veins, it would be expected to approximate the plasma cardiac output. For the normal dog, Sapirstein (137) reports the total cardiac output is 2.82ml. of

blood/second/kilogram of body weight. According to Smith et al. (155) normal dog blood is 53% plasma, so the plasma cardiac output is 1.5ml. of plasma/second/kilogram of body weight. The average body weight of the animals in these experiments was 21.8 kilograms (Table VI). The average plasma cardiac output for these animals is therefore 32.6ml./sec. This is of the same order of magnitude as the rate constant for the exchange between the first two compartments and serves to confirm the anatomical identity attributed to these compartments.

For exchange between the third and fourth compartments, the average rate constant was 2.8ml./sec. In this analysis, this rate constant is thought to reflect the velocity of penetration of creatinine into the cellular elements of the body. In their two compartment model for the distribution of creatinine in the dog, Sapirstein et al. (138) found that the rate constant representing the exchange of creatinine between the "plasma" (that compartment into which the creatinine is quickly distributed) and the "tissues" (the more slowly equilibrating compartment) was 3.2ml./sec. Similarly, in their two compartment model, based on the assumption that the rate of change of creatinine concentration is proportional to the creatinine concentration gradient existing between the two compartments, Dominguez et al. (36) calculated the average rate constant for exchange between the "plasma" and "tissues" to be 2.5/hour. This figure is mathematically equivalent to K_3/V_4 in Equation 6 in our analysis. Using the average value of V_4 measured in our experiments, the rate constant of Dominguez et al. (36) can be calculated to be 3.3ml./sec. Thus, both the rate constants previously determined for the penetration of creatinine into the cells are of similar magnitude to the rate constant computed in the four compartment model used in the present analysis.

G. Discussion

In order to assess the validity of the model proposed in this chapter, several criteria must be considered. One of the first, and most obvious, is whether or not the theoretically predicted and experimentally observed responses were similar. When no systematic deviations exist between the calculated and observed values, the model is considered consistent (16). Using this standard of judgment, on the basis of the data presented, the models of both the sampling system and the animal are sound, as well as consistent. Since the sampling system model was an arbitrary one created only to mimic the characteristics of the response of the real sampling system, the fulfillment of this criterion is sufficient for this model. This is not the case, however, with the compartmental model of the animal. Berman and Mones (16), when referring to biological models, have stated that, "An arbitrary model chosen from a whole class of models for the mere purpose of data fitting is meaningless." This sentiment was echoed by Sharney et al. (147) saying that, "Models should elucidate metabolic processes beyond the mere numerical representation of the experimental data." As previously suggested (16), it is not possible to predict the form of a model on the basis of the concentration data alone, because any model compatible with the data can always be interpreted as a subsystem of a model of higher order. On the basis of the data, however, one can derive a model of minimal order; a model of a lower order would not be sufficient to predict the data. It was shown in this study that a model of less than fourth order would not be sufficiently flexible to fit the experimentally observed data. The compartmental model described above was not an arbitrary one, but was based upon previous knowledge

concerning the anatomical composition of the animal in addition to an impression of the functional characteristics of each of the compartments. The first model proposed was suggested by the division of the body water into three anatomically discrete volumes (plasma, interstitial fluid, and intracellular fluid).[7] It was found that it was necessary to modify and refine the model during the course of the simulation of the animal experiments. But, within the content of the previous functional knowledge of the animal, the modifications necessary for the simulation were intelligible and informative. This simulation demonstrated that the anatomical plasma volume was not functionally discrete, but actually behaved as if it were composed of a fast and slow component roughly analogous to the arterial system which exchanges with the capillary and venous spaces. Furthermore, it was found that the circulating plasma very rapidly exchanged material with a fluid space approximately equivalent in size to the plasma volume. This graphically demonstrates the differencebetween anatomical and functional volumes.

A second criterion that may be used to ascertain the validity of the compartmental model is the standard of uniqueness. That is, could the data have been equally well fitted by another model? "When the fit is good, but the claculated values of the parameters show large uncertainties, the model is nonunique."(15). In practice, it was found for an individual simulation, that the form of the computed arterial creatinine concentration curve was quite sensitive to variations in all of the volume and rate constant parameters. As would be expected, the parameters for the proximal compartments were more exquisitely sensitive to variation than those pertaining to the distal compartments. The model was, therefore, unique for each of the experiments. When the entire

series of simulations is considered, however, the rather large standard deviations in some of the parameters (Tables VI and VII) would suggest that the model is not entirely unique. A portion of this variation, however, was due to the biological variation between the animals rather than to lack of uniqueness in the model. The degree of variability between the animals is reflected in the standard deviations of the body weight and urinary excretion rate constant (Tables VI and VII). With regard to uniqueness in models, H. Warner (167) has stated that, "When an equation is found which will describe the system, its value must be judged on the basis of its ability to describe the system under all circumstances. The equation should have a minimum number of parameters and each parameter should be sensitive to changes in a particular system characteristic. If these two criteria are satisfied, the question of uniqueness of the equation is of no concern." It is this author's impression that the model proposed for the animal is unique in that the same degree of agreement could not be obtained between the computed and observed plasma concentration curves with a model with a fewer number of parameters. Whether or not the observed data could be equally well predicted by four compartmental models arranged differently was not determined in this analysis.

It is evident that the validity of the model is largely determined by soundness of the assumptions upon which it is based. The rationale and basis for all of the assumptions made in this analysis are presented in the section "Assumptions and Definitions". Since by their very nature the assumptions tend to simplify the biological situation in order to make it amenable to mathematical analysis, the model is a simplified reflection of the physiological status of the animal. The mathematical

model approximates reality only to the extent that the assumptions represent the true situation existing in the animal.

Finally, the validity of the model is limited by the reliability of the data upon which it is based, in addition to the accuracy of the techniques by which those data are processed. The accuracy and precision of the creatinine measurement method used in these experiments is discussed in Chapter I. The digitalization and analog conversion of these data introduce a small, random, and generally insignificant error into the data used for the model testing. Figure 18 presents the effect of a single degitalization error. The tape recorder is accurate in recording voltages to within 0.1%. The decade potentiometers of the analog computer are accurate to three significant figures, but on occasion only two significant digits were used (see Table IV). The analog computer amplifiers were tested and found to be accurate to within 0.1% over the full range of amplification, which was -100 volts to +100 volts. Drift in the amplifiers was negligible. By comparing the observed and computed concentration curves on separate beams of a multiple channel oscilloscope, paralax errors were virtually eliminated (174). It is evident that the technical sources of error in this simulation are multiple, but by using components of high quality and stability, these are minimized as much as possible. A quantitative estimate of the accuracy of the derived parametric values, based upon the estimated errors from the various components, is beyond the scope of this presentation. Suffice it to say that it is felt that the biological variation of the experimental subject (which was assumed to be zero) limits the accuracy of this analysis to a greater extent, than the technical collection and processing of the data. The data available limit the validity of the model also in that variations

in the plasma concentrations can be quite insensitive to concentration changes in the distal compartments (151). It is hoped that this restriction is partially obviated by analyzing those experiments that showed rather large fluctuations in the $\left[Cr\right]_p$.

The construction of mathematical models serves the useful function of directing further research into the system being studied. Several additional experiments are suggested by this simulation. As previously emphasized, the model was constructed of functional rather than anatomical compartments. The present study suggests, but does not prove, the anatomical identity of these compartments. Ideally, one would hope to be able to identify the nature of the fluid spaces on each of the functional compartments. Several experiments are suggested that might resolve this question. One experiment that could be easily performed is to measure the creatinine concentration in a peripheral vein during an experiment performed according to the protocol presented in Chapter I. The model predicts that the fluctuations in the creatinine concnetration in the venous system should be a dampened reflection of the arterial $\left[Cr\right]_p$. This type of experiment would provide confirmatory evidence pertaining to the anatomical identity of the second functional compartment predicted by the model. A second experiment suggested by the model would be to measure the creatinine concentration in other anatomical fluid spaces, such as the thoracic duct and cerebrospinal fluid. These data would serve to confirm the anatomical identity of the model compartments. The measurement of tissue creatinine concentrations during experiments similar to the experiments performed in this study, while being technically difficult, would also provide evidence relevant to the exact identity of the components of the compartments detected in this study.

Another type of experiment is suggested by this model. In this analog, it was implied that the exchange between the first two compartments corresponded to the exchange between the arterial and venous plasma volumes. It was also suggested in one of the experiments that vasoconstriction would significantly alter the rate of exchange between the first two compartments. Landis and Pappenheimer (93) previously suggested that the clearance of test materials may be very much smaller during vasoconstriction. This could be confirmed by constructing this model from the data derived in experiments performed on animals treated with vasoconstrictive drugs or on animals with different body temperatures. This would provide direct evidence relating to the anatomical location of the barrier between the first two compartments and also provide a quantitative estimate of the effect of these conditions on the transfer of metabolic substances in the body. In view of the finding that shock slows the distribution of sodium in the body (61), it is suggested that the effects of shock of different types might be quantitatively studied with the model proposed in this chapter.

It was found that the volume of distribution for creatinine was somewhat less than the volume predicted from other tracer experiments. On the basis of the model proposed in this chapter, it is suggested that this discrepancy is due mainly to the relatively small fourth (intracellular) compartment. This was interpreted as the result of the interruption of the femoral arterial circulation to the hind limbs. This simulation, therefore, suggests that experiments be performed on animals with entirely intact circulatory systems in order to ascertain the correctness of this hypothesis.

It would be interesting to see if the model proposed for the

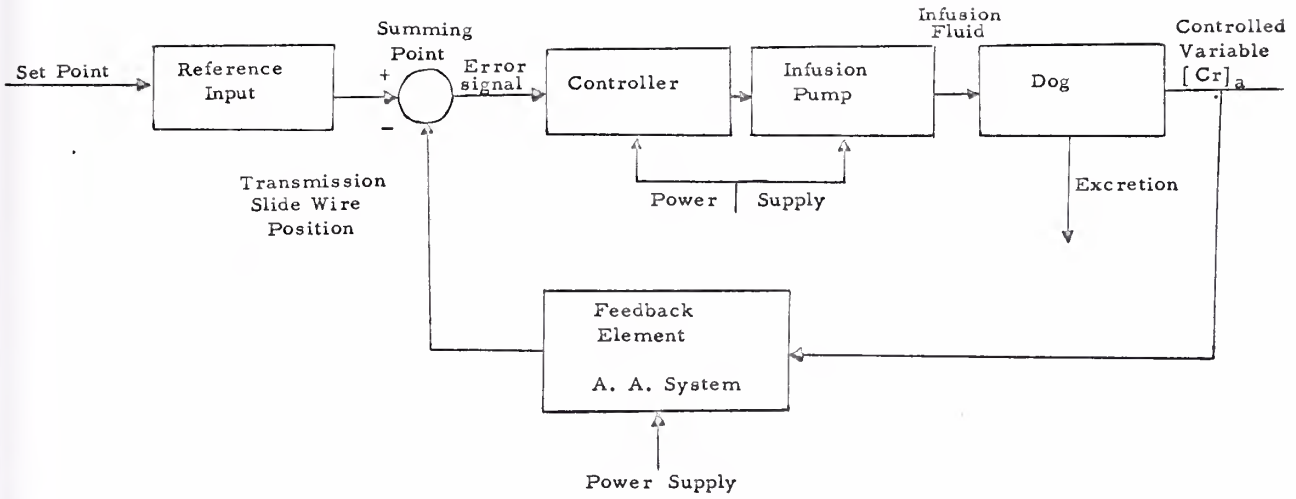
distribution of creatinine could also explain the kinetics of the distribution of other diffusible indicators in the body. It is also suggested that this model might be applied to the study of creatinine kinetics in other animals thereby giving information relevant to comparative physiology. In any event, the model proposed in this study would be a useful starting point in the construction of models of the distribution of many substances in a variety of animals.

A number of interesting physiological observations can be made on the basis of the model for the distribution of creatinine proposed in this study. The fact that it was possible to compute arterial plasma creatinine concentrations which were nearly identical to the observed concentrations implies that the assumptions upon which the model was constructed are, for the most part, correct. These results indicate that the movement of creatinine in the body is mathematically compatible with a diffusion process. As pointed out by Renkin(129), however, diffusion and perfusion processes are intimately associated. It is also indicated that the diffusion into the cellular compartment is slower than the movement of creatinine into any other space and is therefore the rate determining step for the distribution of this substance. This model also supports the hypothesis that the creatinine synthesized in the cellular compartment exchanges with the interstitial space and then into the plasma from whence it is removed from the animal in dircet proportion to the plasma creatinine concentration. It is felt that this model is particularly informative because it characterizes the rapid phase of the distribution in the animal. This phase has not heretofor been analyzed in the models proposed to study canine creatinine distribution.

Aside from the contributions to the understanding of canine physiology, it is felt that the technical aspects of this simulation are noteworthy. This simulation entailed the use of two digital computers and an analog computer. It was demonstrated that the digital computer could be successfully used as a repetative generator. It was further shown that the hybrid computer composed of an on-line digital computer, an analog tape recorder, and an analog computer provides the flexibility and speed necessary to achieve an accurate simulation of both the sampling system and the experimental subject. The on-line digital delay line has not heretofor been described and its advantages of flexibility and rapid access make it a suitable component for use in other experimental analogs. While it is not to be construed that this simulation was primarily a technical exercise, it is suggested that the methods presented here can be adapted to future experimental simulations.

In review, this chapter has presented the computer simulation of the canine experiments described earlier. This simulation entailed the construction of mathematical models for the creatinine sampling system and the experimental subject. An electrical Paynter filter and digital computer delay line were used to create the same distortion and delay in a voltage representing the arterial creatinine concentration as was produced by the AutoAnalyzer system which measured the plasma creatinine concentration. The analog computer model for the dog was founded on the general assumptions of compartmental analysis, in addition to particular assumptions relating to the physiology of creatinine. The equations for this model are presented. The observed rate of creatinine infusion was used as a driving function for the analog of the animal. The parameters of the animal model were manipulated in order to

make the observed and computed arterial plasma creatinine concentrations identical. The computer techniques used to achieve this simulation are described. The observed plasma creatinine concentrations could be successfully predicted by a four compartmental catenary model with infusion into and excretion from the first compartment. In this model, the creatinine moved between the compartments in proportion to the concentration gradient of creatinine that existed between the compartments. The parameters derived from this model are discussed with regard to the physiology involved in the creatinine distribution in the dog. The validity of the models is discussed. It is concluded that the simulation of these experiments contributes information pertinent to the understanding of canine physiology, and also suggests directions for future research, in addition to demonstrating the application of sophisticated computer techniques to the solution of physiological problems.



BLOCK DIAGRAM OF SERVO SYSTEM

Figure 1: BLOCK DIAGRAM OF SERVO SYSTEM

The error signal generated in the servo controller drives an infusion pump which discharges a concentrated creatinine solution into the dog. The AutoAnalyzer, which continuously measures the plasma creatinine concentration, forms the feedback element in this closed-loop control system.

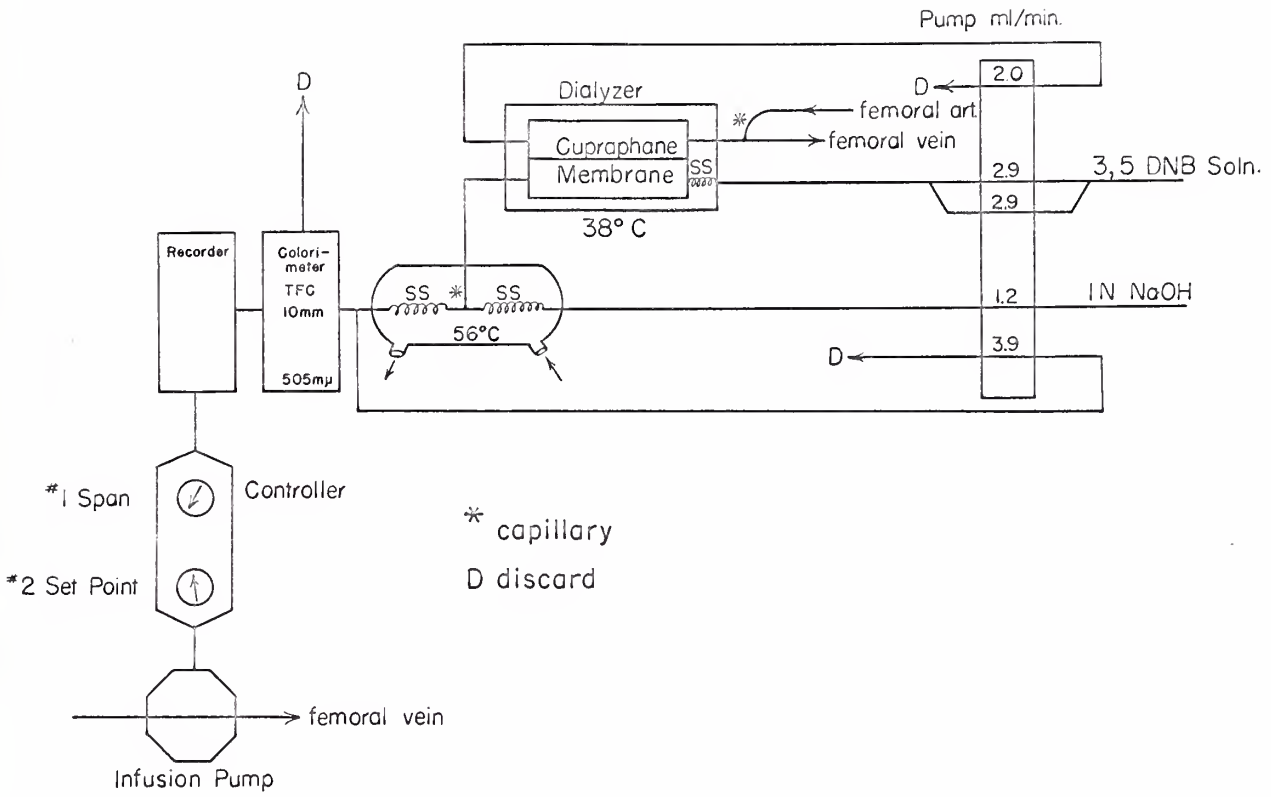


Figure 2: DIAGRAM OF CHEMICAL METHOD TO MEASURE CONTINUOUSLY PLASMA CREATININE CONCENTRATION

The pump, dialyzer and recorder are standard parts of the Technicon AutoAnalyzer system. Blood is pumped from an arterio-venous fistula at two ml. per minute for analysis. Mixing coils, labelled SS, have a volume of four ml.

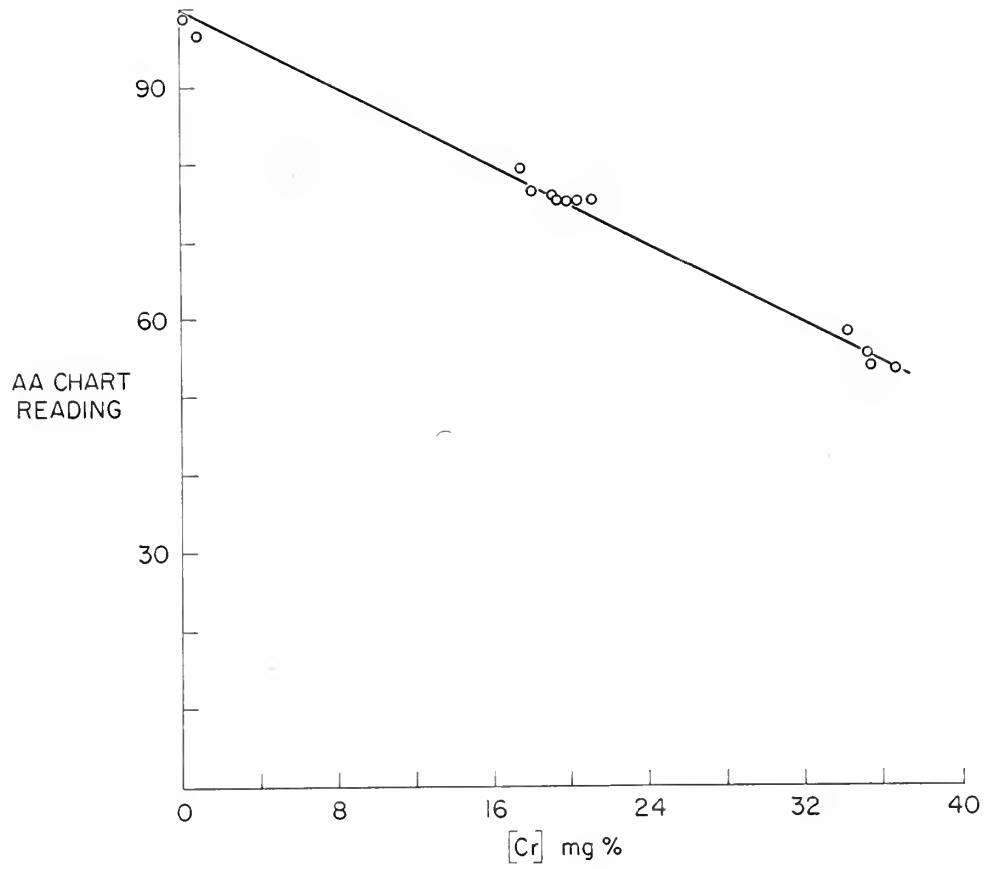


Figure 3: CALIBRATION OF AUTOANALYZER METHOD FOR MEASURING PLASMA CREATININE CONCENTRATION

The ordinate of this graph represents the Auto-Analyzer pen deflection occurring when whole blood is sampled. The abscissa represents the plasma creatinine concentration which was measured by an AutoAnalyzer method sampling the supernatant of the centrifuged whole blood samples. The calibration line was computed by the method of least squares.

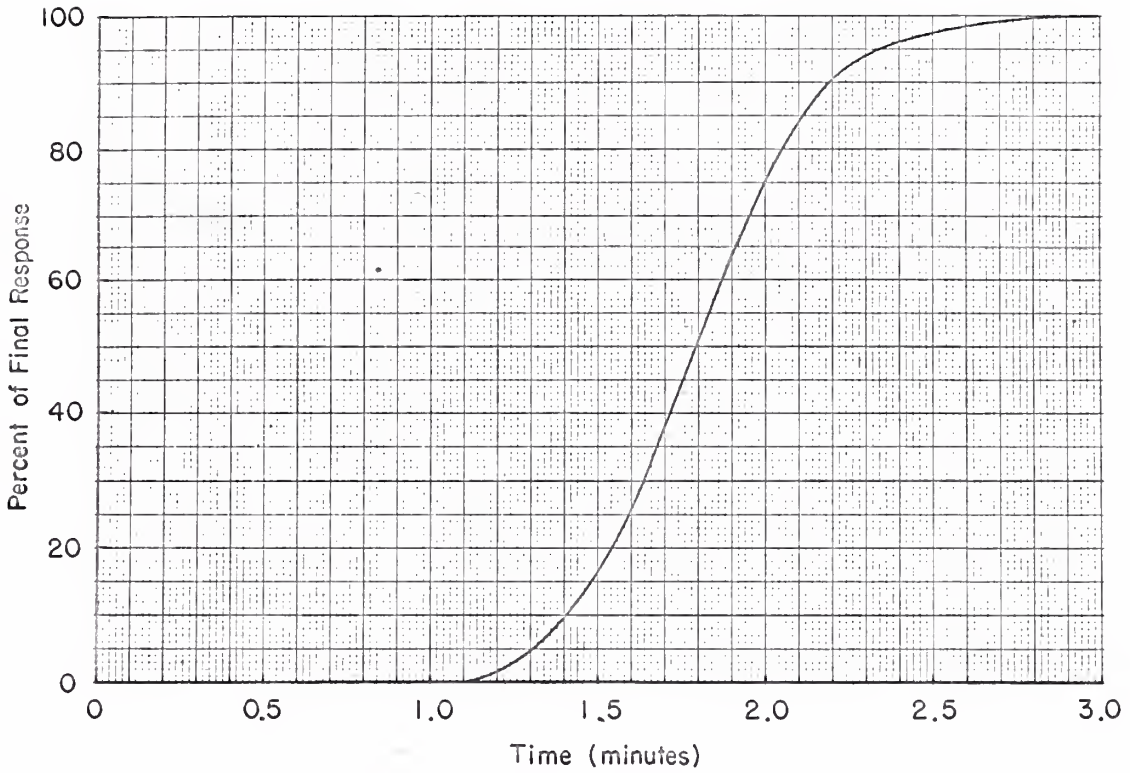


Figure 4: RESPONSE OF THE AUTOANALYZER SYSTEM TO A STEP INPUT OF CREATININE

At time zero, the AutoAnalyzer began to pump from a concentrated creatinine solution.

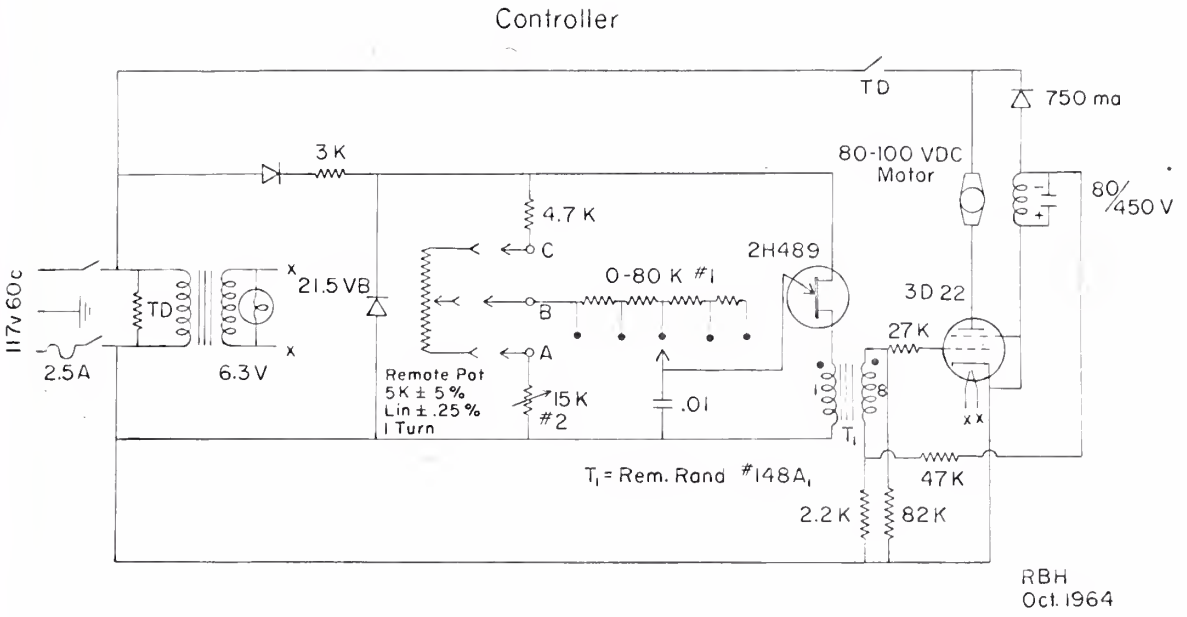


Figure 5: CIRCUIT DIAGRAM OF THE ELECTRONIC CONTROLLER

The remote potentiometer was mechanically linked to the AutoAnalyzer pen recorder. Switch #1 is the span control. Potentiometer #2 is used to control the set point. The 1/20 H.P. motor drives the infusion roller pump through a variable gear transmission.

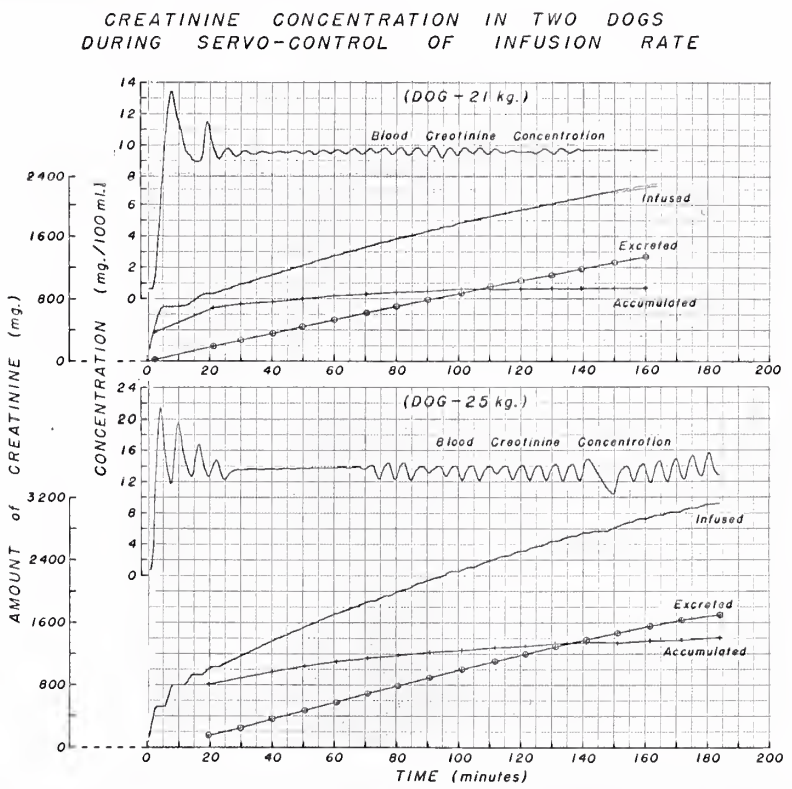


Figure 6: RESPONSE OF TWO DOGS TO SERVO CONTROL OF CREATININE INFUSION RATE

The graphs present the plasma creatinine concentration as well as the time course of creatinine infusion, excretion and accumulation. These graphs were constructed by the CALCOMP on-line plotter under the control of the IBM 1620 computer. The dog in the upper panel is number 2374 and dog number 1384 is presented in the lower panel.

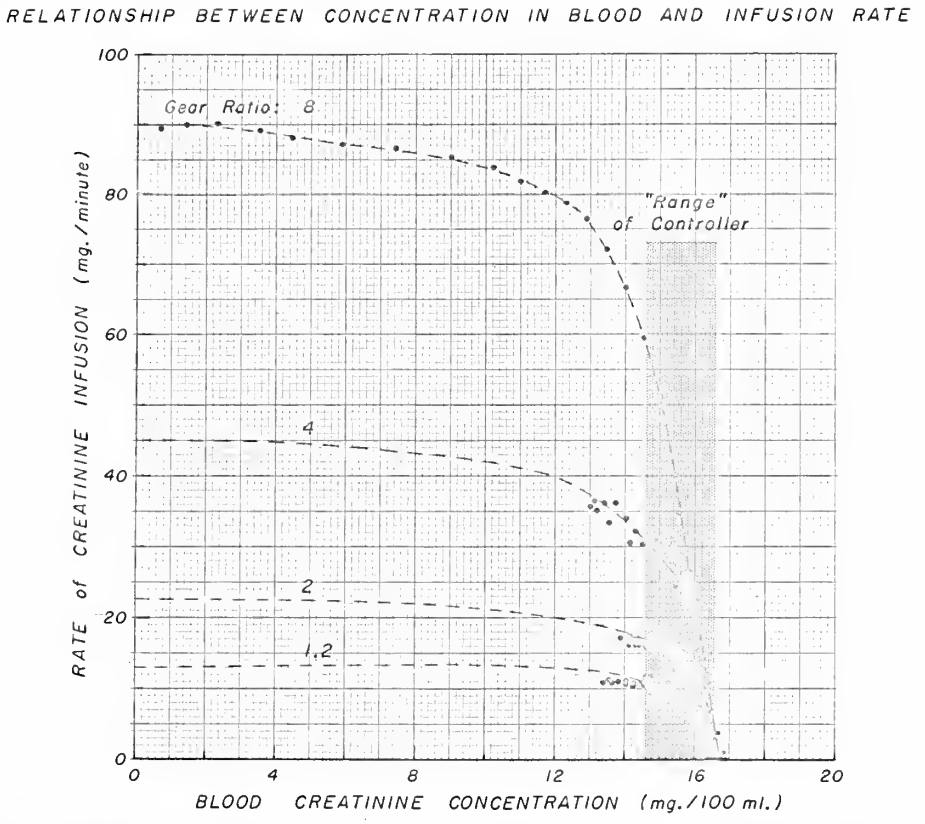


Figure 7: RELATIONSHIP OF THE PLASMA CREATININE CONCENTRATION AND THE INFUSION RATE

The rate of creatinine infusion was computed every 20 seconds during the experiment on dog number 1484. The CALCOMP on-line plotter, under the control of the IBM 1620 computer, constructed this graph by pairing these rates of infusion with the $[Cr]_p$ during the corresponding 20 second interval. These were plotted for the various pump: motor transmission gear ratios used during the experiment.

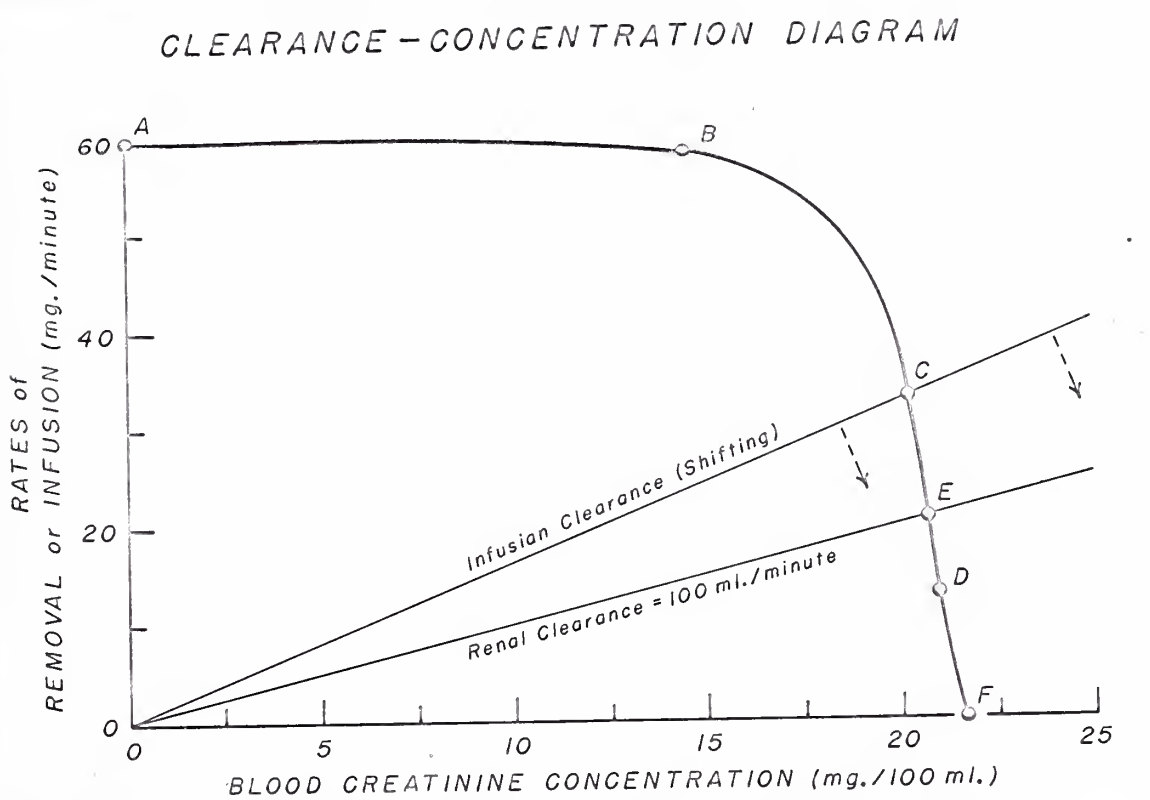


FIGURE 8: THEORETICAL CLEARANCE – CONCENTRATION DIAGRAM

The curve represents the transfer function of the servo system relative to the creatinine concentration. The straight lines represent the clearances of substances which are removed by glomerular filtration.

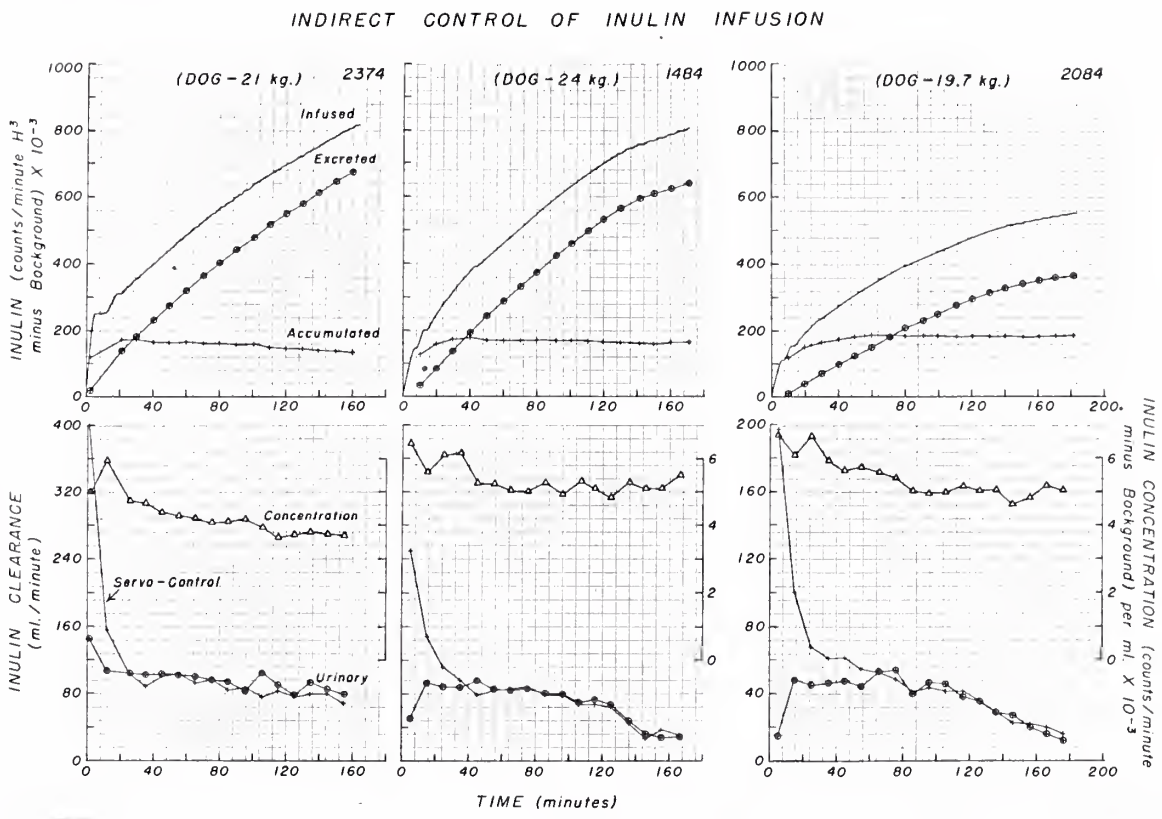


FIGURE 9: INDIRECT CONTROL OF INULIN CONCENTRATION IN THREE DOGS

The upper panels describe the fate of the infused inulin. The lower panels present the time course of the concentration as well as a comparison of the servo and urinary clearances.

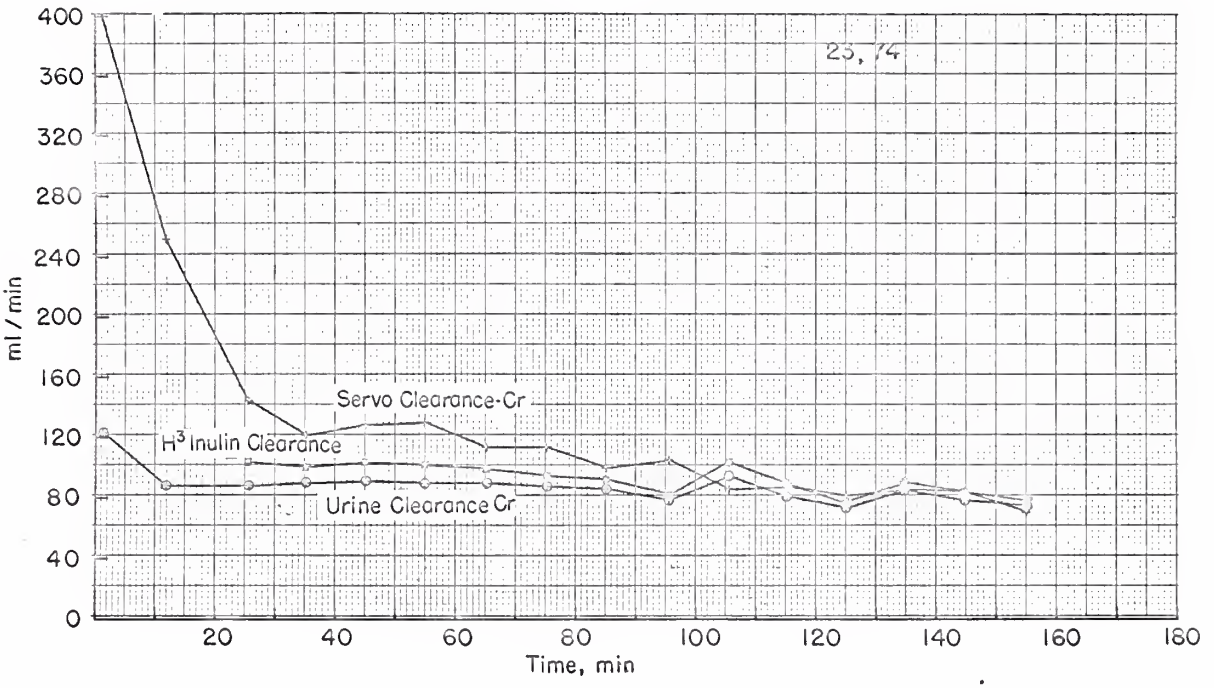


FIGURE 10: Comparison of the Servo and Excretion Clearances of Creatinine with the Excretion Clearance of Inulin in Dog 2574.

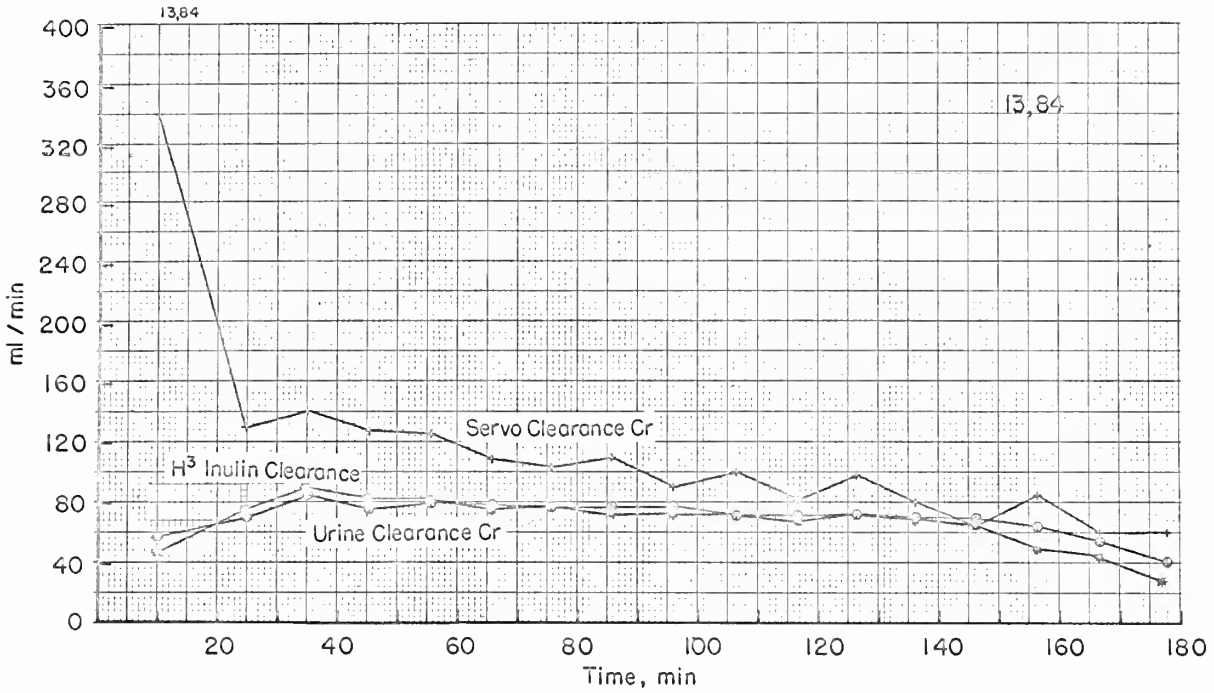


FIGURE 11: Comparison of the Servo and Excretion Clearances of Creatinine with the Excretion Clearance of Inulin in Dog 1384.

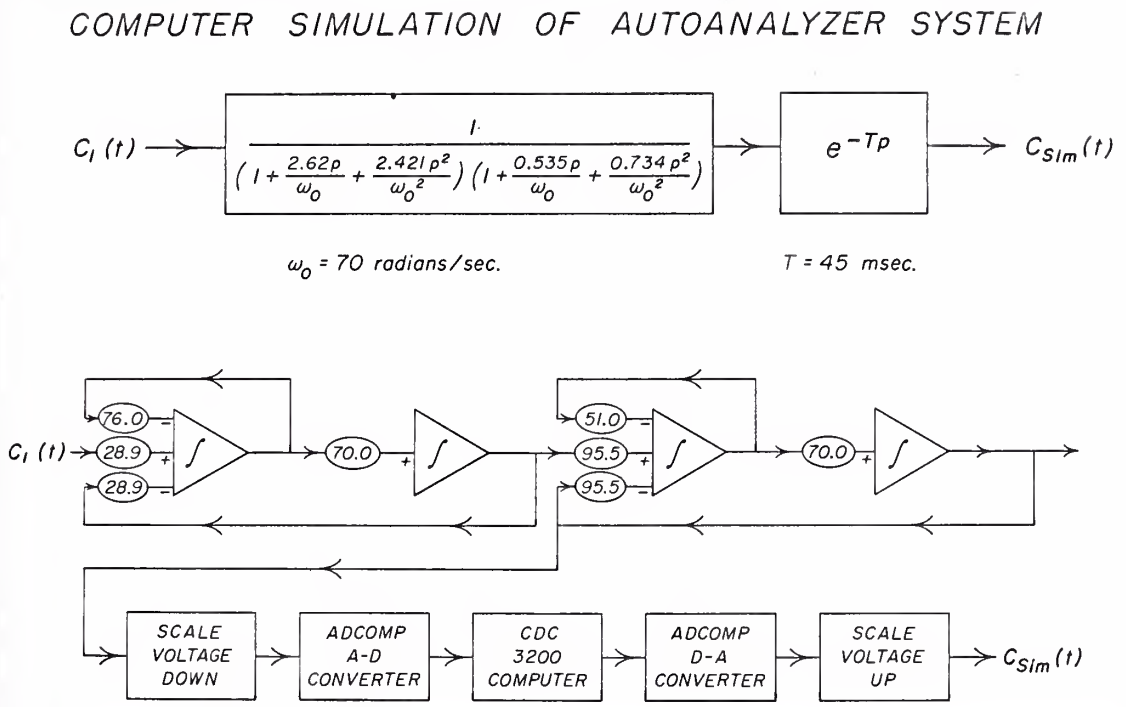


Figure 12: COMPUTER SIMULATION OF AUTOANALYZER SAMPLING SYSTEM

Top panel: A voltage representing the plasma creatinine concentration is first dispersed by a Paynter filter and then delayed by a digital computer, in order to yield a voltage which corresponds to the observed plasma creatinine concentration.

Bottom panel: Detail of the computer arrangement of Paynter electrical filter and digital delay system.

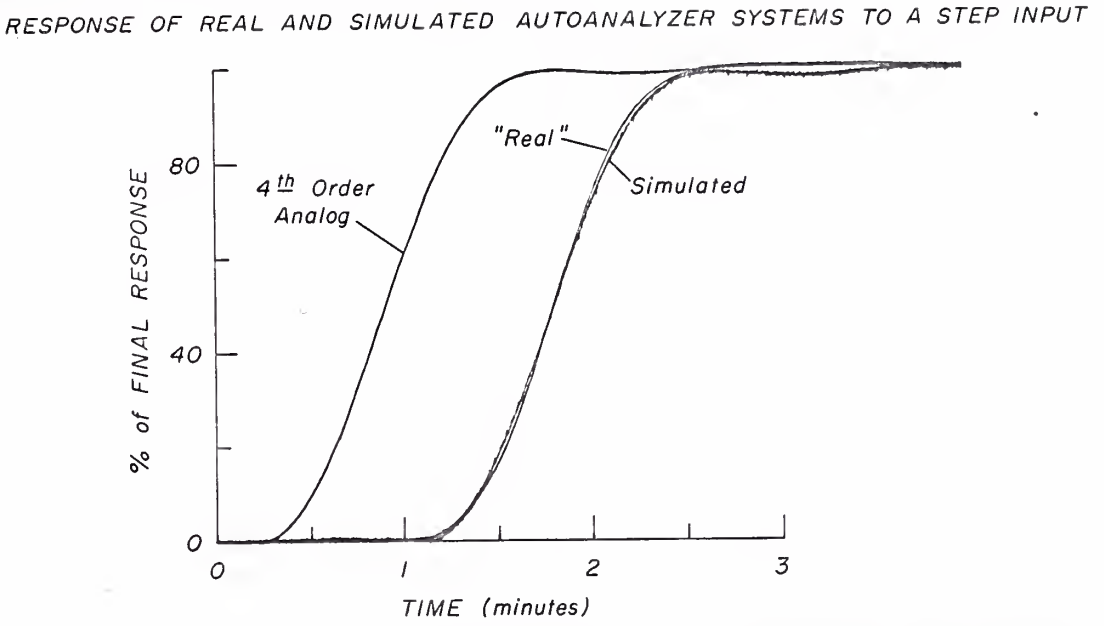


Figure 13: RESPONSE OF REAL AND SIMULATED AUTOANALYZER SYSTEMS TO A STEP INPUT

The curve on the left represents the output of the Paynter filter responding to a step input of voltage. The "simulated" curve is the "4th Order Analog" curve after it has been passed through the digital delay system. This delayed curve is to be compared with the "Real" curve, which is the observed response of the AutoAnalyzer system to a step input of creatinine, as it was reconstructed on the electrical function generator.

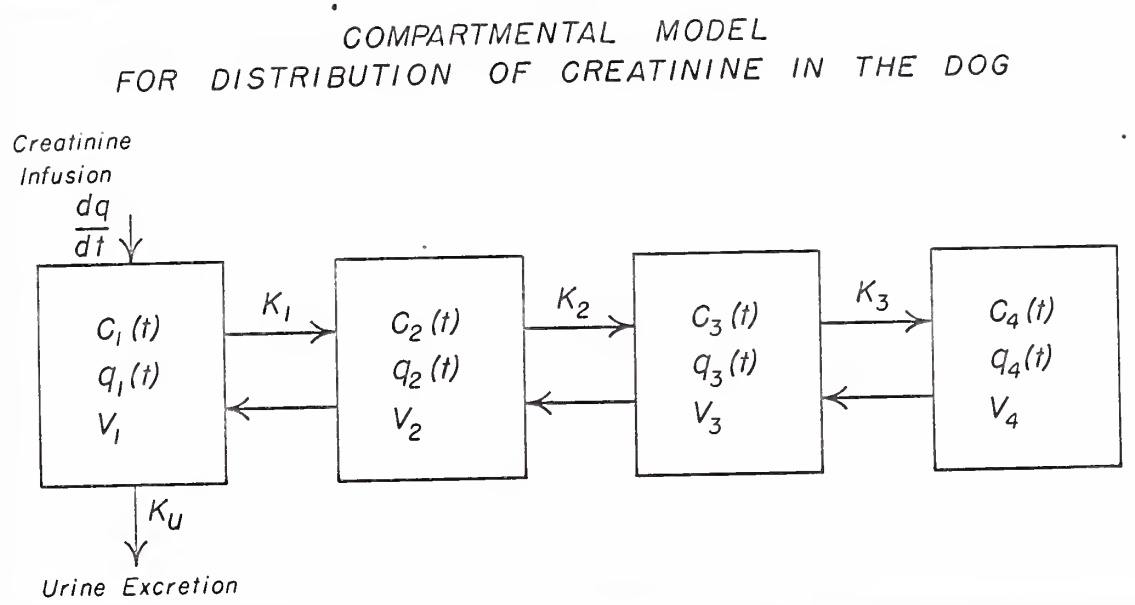


Figure 14: FOUR COMPARTMENT CATENARY MODEL USED TO SIMULATE THE DISTRIBUTION OF CREATININE IN THE DOG

Creatinine is infused into and excreted from the first compartment. Rate constants into and out of a compartment along a given route are assumed to be equal. Each compartment is characterized by a creatinine concentration, C(t), a volume, V, and a quantity of creatinine, q(t).

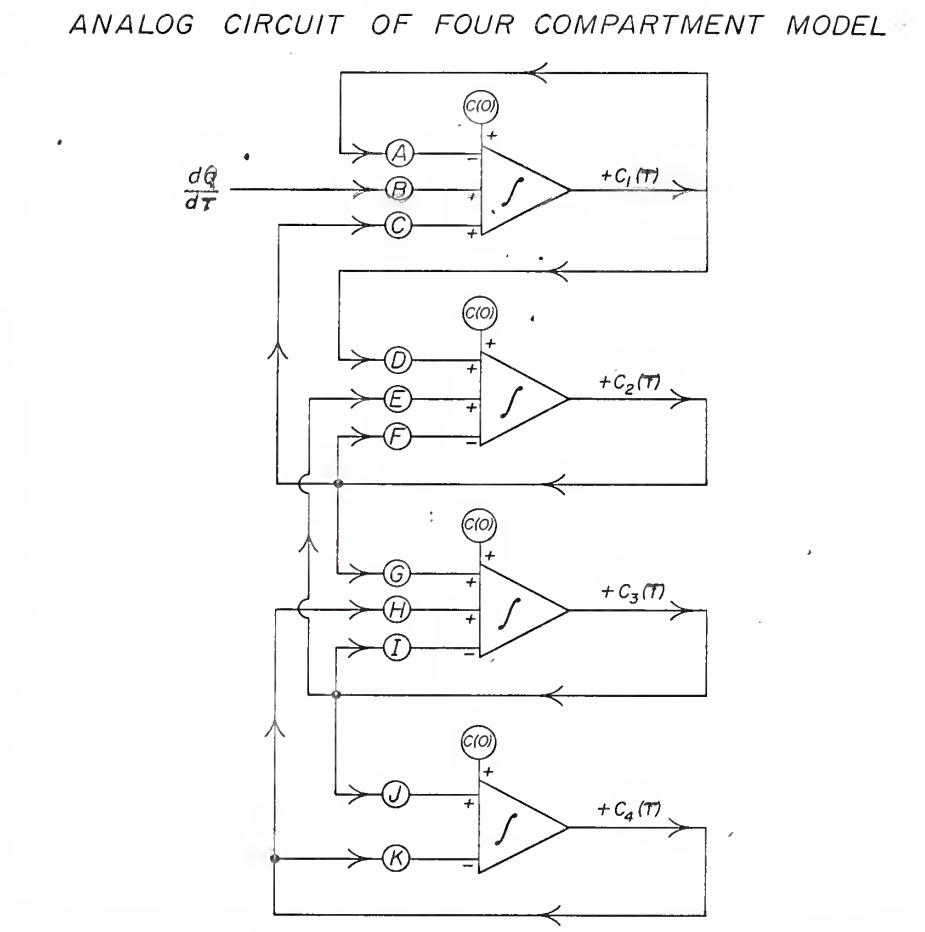


Figure 15: ANALOG COMPUTER CIRCUIT OF FOUR COMPARTMENT CATENARY MODEL WITH INFUSION INTO AND EXCRETION OUT OF THE FIRST COMPARTMENT

The numerical and mathematical values of the potentiometers in the different experiments are given in Table IV.

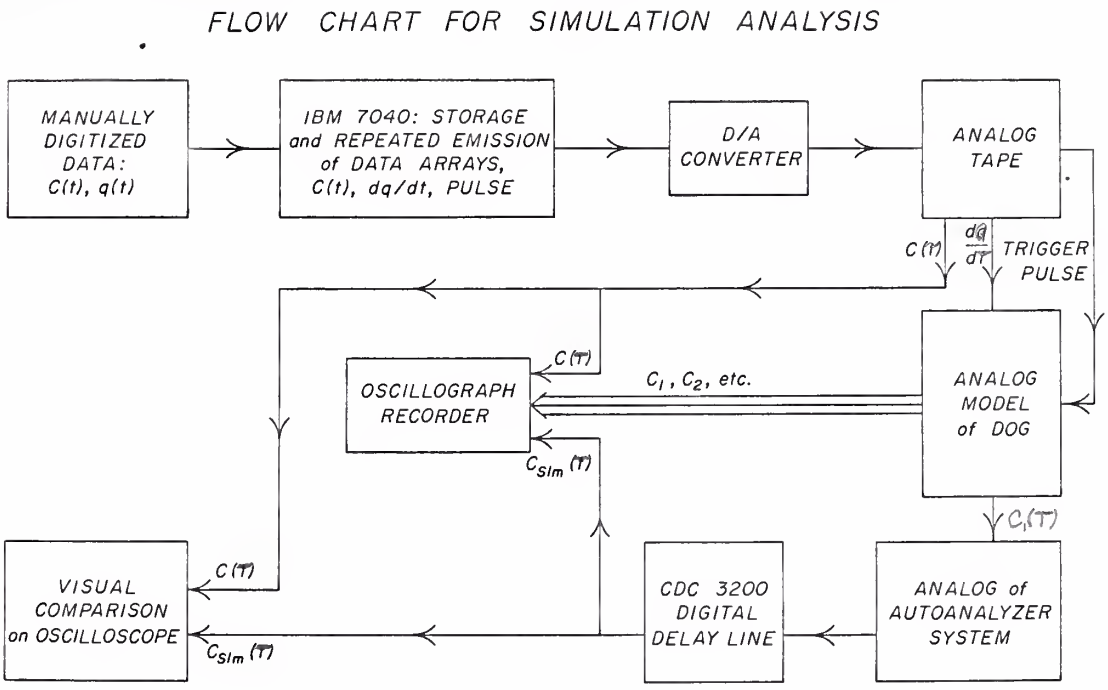


Figure 16: FLOW CHART OF COMPUTER METHOD FOR SIMULATING CREATININE DISTRIBUTION

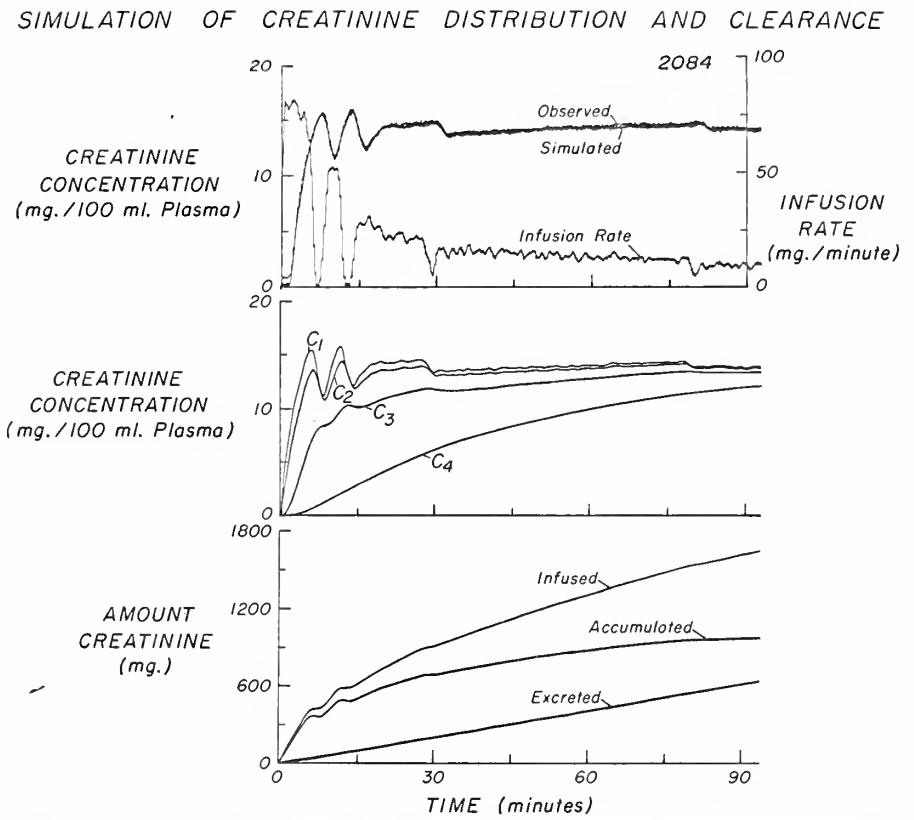


Figure 17: RESULTS OF COMPUTER SIMULATION OF CREATININE DISTRIBUTION IN DOG 2084

Top Panel: The agreement between the computed and the observed creatinine plasma concentrations is presented along with the voltage which represents the rate of creatinine infusion.
Middle Panel: The creatinine concentration in each of the four compartments is presented.
Bottom Panel: The computed amounts of creatinine infused, excreted, and accumulated in the model are presented.

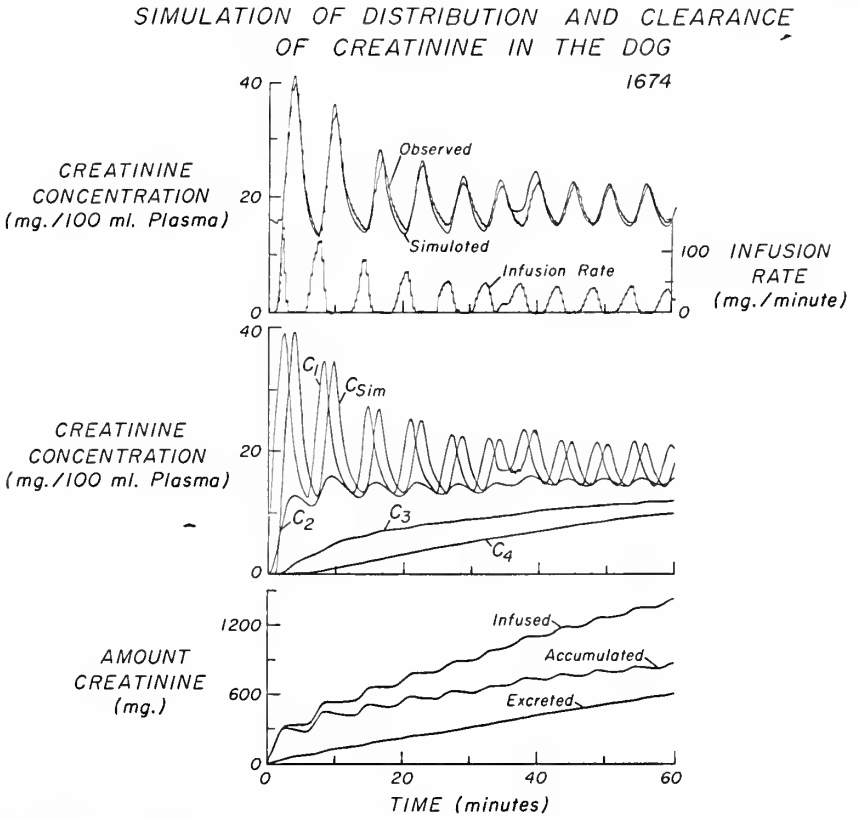


Figure 18: RESULTS OF COMPUTER SIMULATION OF CREATININE DISTRIBUTION IN DOG 1674

The three panels are of the same significance as the three panels in Figure 17. Note that the middle panel shows the effect of the sampling system analog on C_1 to produce C_{sim}. A digitalization error at about 55 minutes produced a small error in the infusion rate and this, in turn, was reflected in the concentrations of creatinine in the model.

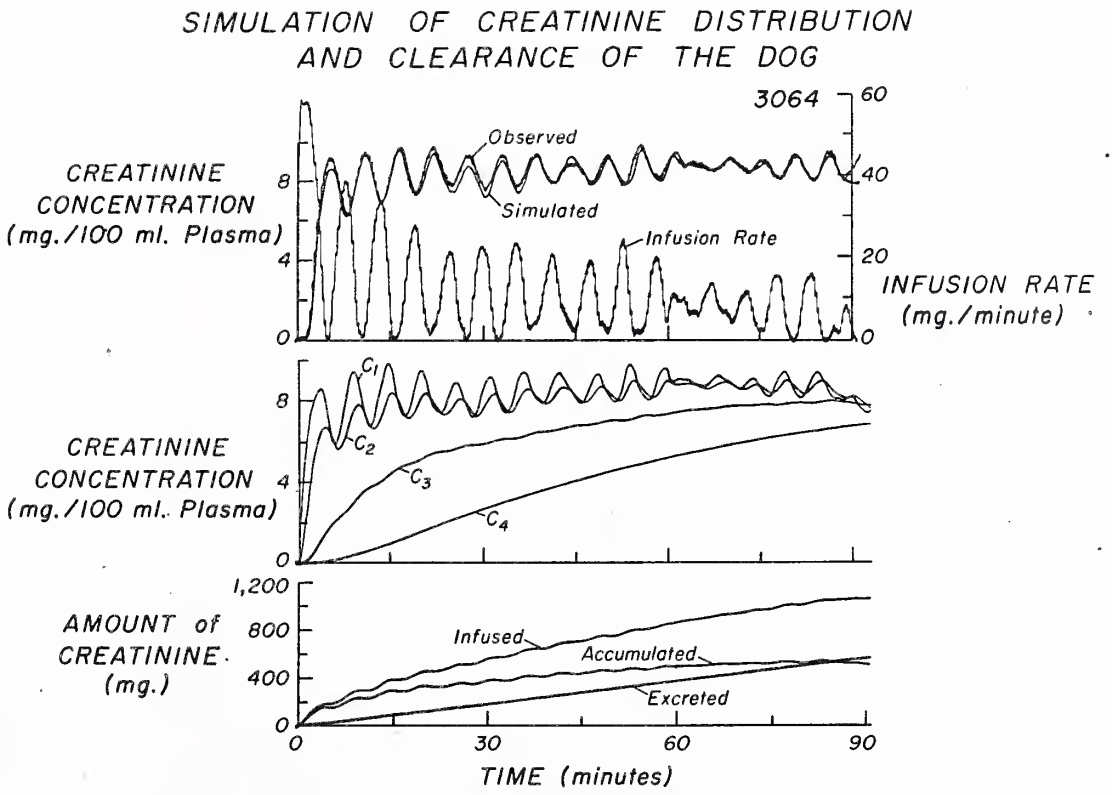


Figure 19: RESULTS OF COMPUTER SIMULATION OF CREATININE DISTRIBUTION IN DOG 3064.
The panels are of the same significance as those in Figure 17.

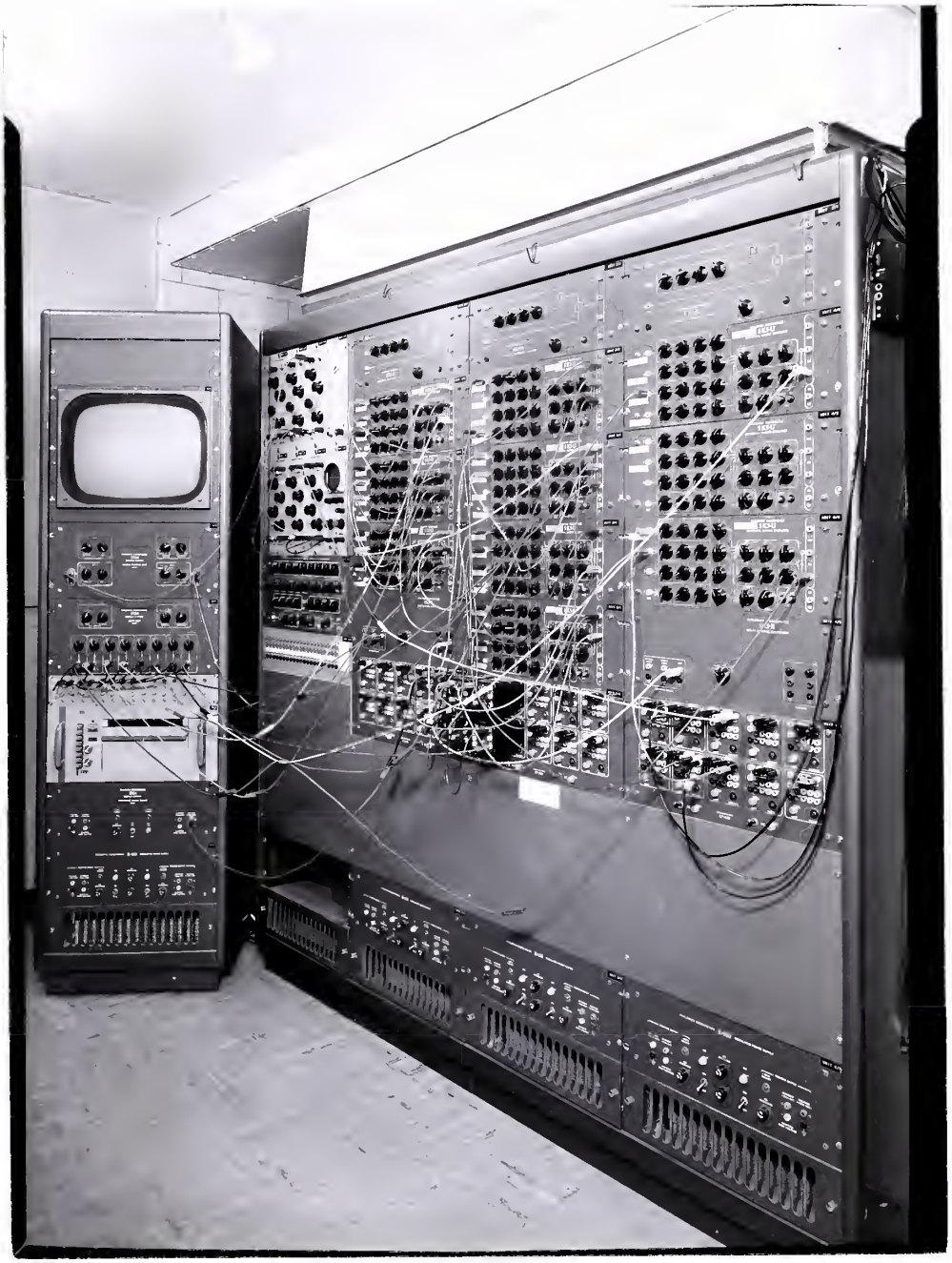


Figure 20: PHOTOGRAPH OF THE ANALOG COMPUTER PROGRAMMED FOR THE SIMULATION OF CREATININE DISTRIBUTION EXPERIMENTS

The black lines on the left transmit the data from the analog tape recorder. The analog computer is programmed with the compartmental model of the experimental subject and the electrical analog of the sampling system. The eight channel oscilloscope is on the left and immediately below it is the photographic recorder.

Figure 30:

TABLE I: URINARY CREATININE CLEARANCE (ml/min)
SERVO CREATININE CLEARANCE (ml/min)

TIME AT END URINE COLL. (min.)	DOG 3064	DOG 674	DOG 774	DOG 1374	DOG 1674	DOG 1384	DOG 2374	DOG 1484	DOG 2084
10		47/586		52/617				75/680	23/586
20	68/178	48/183	36/420	56/234	41/129	63/428	97/315	77/216	41/174
30	56/118	50/146	57/176	73/183		70/137	89/133	81/171	42/123
40	67/122	50/103	58/143	69/158	48/98	85/147	91/117	81/145	44/102
50	65/119	50/87	60/124	73/145	53/96	75/131	92/124	82/106	46/97
60	57/115	49/71	60/112	72/129		80/129	91/124	82/116	44/81
70	87/91	45/78	43/97	74/116		78/111	91/107	80/113	53/79
80	98/80	44/71	25/76	74/118	49/75	76/104	89/109	77/113	54/71
90	72/70	40/57	0/36*	76/119	58/71	76/112	87/97	73/104	42/57
100	47/76	38/47	0/34	75/96		77/91	80/100	68/96	47/60
110	84/235 **	34/57	0/26	**	45/51	72/103	95/84	65/87	51/58
120	85/151	26/47	0/20	72/213		71/84	82/85	66/83	40/58
130	76/133	18/22	0/18	72/139	32/32	73/97	75/79	60/75	36/48
140	72/128	11/28	0/15			71/78	87/82	51/57	30/39
150	76/113	6/16		74/118	55/44	67/62	79/81	31/34	26/29
160	91/94		0/12		37/42	64/87	76/86	27/45	22/28
170			0/9	74/97		54/62		31/33	19/26
180					29/36	41/51			15/20
190									
200					21/25				

* ureters tied

** increased set point concentration

TABLE II

FLUID BALANCE DURING EXPERIMENTS

DOG NUMBER	BODY WEIGHT Kg.	MINUTES DURATION OF EXPERIMENT	ml. REMOVED BY A.A.	ml. OF URINE	TOTAL ml. REMOVED	TOTAL ml. INFUSED	NET ml. FLUID BALANCE
3064	21	160	-320	-490	-810	+549	-261
674	16.7	150	-300	-381	-681	+432	-249
774	16.3	170	-340	-356	-696	+603	- 93
1374	20	169	-338	-1580	-1918	+1340	-578
1674	19.1	206	-412	-735	-1147	+615	-532
2374	21	160	-320	-544	-864	+293	-571
1384	25	184	-368	-700	-1068	+413	-655
1484	24	171	-342	-749	-1091	+400	-691
2084	19.7	183	-366	-428	-794	+284	-510

TABLE III

TABLE OF FLUID BALANCE DURING

EARLY PART OF EXPERIMENTS

EXPERIMENT	MINUTES OF SIMULATION	ml REMOVED BY AA	ml OF URINE	TOTAL ml REMOVED	TOTAL ml INFUSED	NET ml FLUID BALANCE
3064	60	-120	-94	-214	+173	-41
1374	60	-120	-426	-546	+503	-43
1674	90	-180	351	-531	+309	-222
2374	60	-120	-209	-329	+174	-155
1484	60	-120	-349	-469	+228	-241
2084	60	-120	-134	-254	+175	-79

TABLE IV

VALUES OF ANALOG COMPUTER POTENTIOMETERS FOR SIMULATION OF CREATININE DISTRIBUTION IN THE DOG

POTENTIOMETER	EXPRESSION REPRESENTED BY POTENTIOMETER	POTENTIOMETER VALUE IN EXPERIMENT				
		3064	1674	1384	1484	2084
A	$(K_1+K_u)/V_1X_t$	63.5	13.1	32.6	205	98.0
B	X_c/X_qV_1	25.0	37.5	25.0	33	20.0
C	K_1/X_tV_1	60.0	11.2	30.0	200	96.0
D	K_1/X_tV_2	16.0	3.00	7.5	50.0	40.0
E	K_2/X_tV_2	4.0	1.80	4.0	10.0	10.0
F	$(K_1+K_2)/X_tV_2$	20.0	4.80	11.5	60.0	50.0
G	K_2/X_tV_3	2.00	1.12	4.00	5,00	5.00
H	K_3/X_tV_3	0.80	1.12	1.50	1.50	1.50
I	$(K_2+K_3)/X_tV_3$	2.80	2.24	5.50	6.50	6.50
J	K_3/X_tV_4	0.62	0.73	0.63	0.70	0.64
K	K_3/X_tV_4	0.62	0.73	0.63	0.70	0.64

Potentiometer letters refer to Figure 15.

Mathematical expressions represented by potentiometer refer to Equations (10) to (13).

TABLE V

SCALE FACTORS AND INITIAL VALUES FOR COMPUTER SIMULATION OF CREATININE DISTRIBUTION IN THE DOG

CONSTANT	VALUE IN EXPERIMENT				
	3064	1674	1384	1484	2084
X_c	500	250	250	500	500
X_q	1/20	1/60	1/60	1/20	1/20
X_t	1/1200	1/900	1/1200	1/1200	1/1200
C_o volts	0.1	0.9	1.7	2.7	4.4

TABLE VI

COMPUTED COMPARTMENTAL VOLUMES FOR MODEL OF CREATININE DISTRIBUTION IN THE DOG

EXPERIMENT	BODY WEIGHT Kg	V_1 ml	V_1 %BW	V_2 ml	V_2 %BW	V_3 ml	V_3 %BW	V_4 ml	V_4 %BW	V_{total} ml	V_{total} %BW
3064	21.0	400	1.90	1500	7.14	3000	14.3	3870	18.4	8770	41.7
1674	19.1	400	2.10	1490	7.80	2390	12.5	3680	19.3	7960	41.7
1384	25.0	600	2.40	2400	9.60	2400	9.60	5710	22.8	11110	44.4
1484	24.0	300	1.26	1210	5.05	2420	10.1	5190	21.6	9120	38.0
2084	19.7	500	2.54	1200	6.10	2400	12.2	5620	28.5	9720	49.3
AVERAGE	21.8	440	2.04	1560	7.14	2530	11.7	4810	22.1	9340	43.0
STANDARD DEVIATION	2.33	102	0.45	438	1.55	534	1.70	866	3.54	1050	3.74

Volumes are expressed in milliliters and as percent of body weight.

TABLE VII

RATE CONSTANTS FOR FOUR COMPARTMENT MODEL FOR DISTRIBUTION OF CREATININE IN THE DOG

EXPERIMENT	K_y	K_1	K_2	K_3
3064	1.17	20.0	5.00	2.00
1674	0.83	4.98	2.98	2.98
1384	1.30	15.0	8.00	3.00
1484	1.26	50.5	10.1	3.03
2084	0.83	40.0	10.0	3.00
AVERAGE	1.08	26.1	7.21	2.80
STANDARD DEVIATION	0.21	16.7	2.81	0.40

Rate constants are expressed as milliliters/secpnd.

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